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Placental Transmission

III. The Amino-Acids, Non-Protein Nitrogen, Urea, and Uric Acid in Fetal and Maternal Whole Blood, Plasma and Corpuscles

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A CRITICAL survey of the available literature dealing with the placental transmission of the total non-protein nitrogen and the amino-acids, as determined by analysis of simultaneously collected samples of fetal and maternal blood, revealed certain data, which are not consistent with other well recognized facts. It was, therefore, decided to repeat this work in the hope of obtaining more uniform results, or of demonstrating a peculiar biological characteristic in the blood of pregnant women.

Morse (1) found the amino-acids consistently higher in the plasma of the fetus than in that of the mother, but demonstrated the same difference in only 70 per cent of his cases when whole blood was used for analysis. Murlin (2) quotes Bock as having found these substances uniformly higher in the fetal whole blood, whereas Morel and Mouriquand (3) could detect no essential difference.

If Morse's observations on plasma are correct, Bock's figures for whole blood should be accepted, since the corpuscles are generally conceded to be richer in amino-acids than the plasma, and fetal blood always has more cells per unit volume than the maternal blood. If, however, we are to accept Morse's figures for the whole blood, as well as for plasma, we must assume that the corpuscles of the pregnant woman are occasionally so altered that they contain less amino-acids than the plasma, for otherwise the maternal blood could never contain more of these substances than the fetal blood.

Moreover, if Morse's plasma and Bock's whole blood observations are accepted, the total non-protein nitrogen must be always higher in the fetal than in the maternal blood, unless there is a compensatory difference in the concentration of some other nitrogen-containing blood constituents. None of the work on total non-protein nitrogen has shown consistent results: Thus, Landsberg (4) found the plasma values generally, but not always, higher on the fetal side of the placenta, while Zangemeister and Meissl (5) had the opposite experience with serum. Slemons and Morriss (6), Morel and Mouriquand (3), and Howe and Givens (7) could determine no consistency in the whole bloods and in general came to the conclusion that the total non-protein nitrogen was

not essentially different in the two circulations.

It is quite obvious that these various results are not in harmony, and that the whole subject needs further investigation. Consequently, we have examined twenty specimens of fetal and maternal blood and have analysed both the whole blood and plasma for total non-protein nitrogen and the amino-acids. The plasma volume per cent was determined in each sample by hematocrit readings and the corpuscular content of the various substances was calculated therefrom. The urea and uric acid were also determined, since there are no figures available for fetal and maternal plasma, and since the reported values for whole blood have shown no consistent variation in either direction.

TABLE I

Amino-acid nitrogen

In fetal and maternal bloods

	WHOLE BLOOD		PLASMA		CORPUSCLES		CORPUSCLE VOLUME	
	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal
	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	per cent	per cent
1	7.1	5.7	6.8	4.5	7.5	7.9	48.0	35.1
2	7.8	6.6	7.6	5.2	7.9	8.1	46.6	33.3
3	7.8	5.9	6.1	4.2	9.8	9.5	48.4	31.6
4	9.1	6.5	6.8	5.3	11.0	8.7	49.0	35.5
5	8.0	6.0	5.6	3.9	11.5	9.5	40.0	38.1
6	7.1	5.9	5.5	4.3	9.7	8.7	37.0	35.4
7	8.4	6.4	6.1	4.5	10.8	9.5	47.9	32.7
8	9.2	6.9	6.3	5.0	12.8	9.5	45.3	42.0
9	7.6	6.0	5.5	3.6	7.8	10.3	43.3	36.0
10	9.4	6.4	7.5	4.4	11.3	11.0	50.6	30.0
11	8.4	6.0	5.8	4.0	11.2	9.7	49.3	36.0
12	7.6	5.5	5.9	4.1	9.4	8.1	48.0	34.6
13	8.5	7.0	7.6	5.0	9.5	10.3	45.3	38.0
14	9.1	6.2	6.6	4.5	13.7	8.4	55.3	42.7
15	8.7	6.2	6.3	4.2	10.4	10.0	58.7	34.0
16	8.0	5.3	6.1	4.0	10.6	8.1	41.3	22.0
17	7.0	5.3	6.1	4.6	8.5	7.0	38.7	30.0
18	7.4	5.7	5.0	4.3	9.9	10.0	48.7	24.7
19	9.2	6.0	7.4	4.5	10.5	9.1	60.0	34.2
20	9.0	6.3	7.3	5.0	11.2	9.1	42.7	32.0
Ave..	8.2	6.1	6.4	4.5	10.3	9.1	47.2	34.4

EXPERIMENTAL

The specimens were obtained as nearly simultaneously as possible, the fetal from the cut end of the umbilical cord and the maternal from an arm vein. Coagulation was prevented by the use of lithium oxalate and the determinations were carried out by the methods of Folin (8). Ordinarily the analyses were begun immediately, but samples collected at night were preserved in the refrigerator until the following morning. Duplicates were done throughout and when they failed to agree closely, another pair of analyses were run. Hematocrit readings were made after centrifuging the whole blood for twenty minutes at high speed.

RESULTS AND DISCUSSION

For the sake of clearness, the various substances studied are reported in separate tables, each of which is followed by the pertinent discussion.

Amino-acids. The fetal whole blood amino-acid nitrogen varied from 7.1 to 9.4 mg. per 100 cc. and averaged 8.2 mg., while the maternal values ranged from 5.3 to 7.0 mg. and averaged 6.1 mg. In each individual case the fetal sample was higher than the maternal, the difference varying from 1.2 to 3.2 mg., and averaging 2.1 mg. per 100 cc.

The amino-acids of the fetal plasma were also consistently higher than those of the maternal plasma, the

former ranging from 5.0 to 7.6 mg. and averaging 6.4 mg. per 100 cc., while the latter varied from 3.6 to 5.3 mg. and averaged 4.5 mg. per 100 cc. The difference in favor of the fetal sample ranged from 1.2 to 3.1 mg. and averaged 2.0 mg. per 100 cc.

Since whole blood uniformly contained more amino-acids than the plasma, the calculated corpuscle values were also consistently higher. The fetal corpuscles varied from 7.6 to 13.7 and averaged 10.3 mg. amino-acid nitrogen per 100 cc., while the maternal cells ranged from 7.0 to 11.0 mg. and averaged 9.1 mg. per 100 cc. Although the average value for the amino-acids in the fetal corpuscles was thus shown to be appreciably higher than in the maternal corpuscles, the separate samples did not always vary in the same direction, for in 15 cases the fetal values were higher, while in five they were lower.

Folin and Berglund (9) give the range and average of the amino-acid nitrogen content of the whole blood, plasma and corpuscles of 12 normal men as follows:

	RANGE	AVERAGE
Whole blood	5.7– 7.8	6.4
Plasma	4.3– 6.2	5.3
Corpuscles	6.7–10.7	8.2

while Wu (10) gives the following values for plasma and corpuscles in 20 samples of normal blood:

	RANGE	AVERAGE
Plasma	4.0– 7.5	5.52
Corpuscles	7.7–11.4	9.47

By tabulating our results after this fashion we have:

	FETAL		MATERNAL	
	Range	Average	Range	Average
Whole blood	7.1– 9.4	8.2	5.3– 7.0	6.1
Plasma	5.0– 7.6	6.4	3.6– 5.3	4.5
Corpuscles	7.5–13.7	10.3	7.0–11.0	9.1

and by comparing them with those quoted above we find that the fetal values are higher than the accepted normals, while the maternal values are slightly lower. These variations suggest that there is a change in both bloods which tends to accentuate the difference between the two. We have no explanation for the increased amino-acids in the fetal blood and plasma, but have been impressed by the fact that thus far only the materials essential for fetal development—amino-acids, calcium (11) and inorganic phosphates (12)—have been shown to be consistently higher in the fetal than in the maternal blood. This is probably due to "selective absorption" from the maternal blood. At present such a phrase means nothing, although it will no doubt be shown later to represent the working of a definite physico-chemical law, the final appreciation of which will depend upon the careful alignment of all the known facts into a harmonious whole. On the other hand, the slightly lower maternal values may well be the result of the watery dilution which the blood and plasma undergo in normal pregnancy (13).

Our results, then, confirm those of Morse for the plasma and of Bock for the whole blood, and remove all suspicion that the corpuscles suffer any peculiar alteration as regards their capacity for holding the amino-acids

during pregnancy, since it has been demonstrated that the corpuscles always contain more amino-acids than the plasma in this condition as in the non-pregnant individual.

TABLE II

Total non-protein nitrogen

In fetal and maternal bloods

	WHOLE BLOOD		PLASMA		CORPUSCLES		CORPUSCLE VOLUME	
	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal
	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	per cent	per cent
1	31.3	24.8	22.6	17.9	40.8	37.6	48.0	35.1
2	32.0	28.9	27.7	24.1	37.1	38.7	46.6	33.3
3	34.6	31.6	22.9	21.6	47.1	53.1	48.4	31.6
4	41.8	34.4	32.0	25.3	52.0	51.0	49.0	35.5
5	38.0	32.6	28.9	22.7	51.7	48.8	40.0	38.1
6	26.2	23.3	22.5	17.5	32.4	33.9	37.0	35.4
7	46.1	37.7	32.9	27.8	60.5	58.1	47.9	32.7
8	42.5	33.7	25.7	21.7	62.7	50.2	45.3	42.0
9	37.5	27.2	23.2	20.5	56.1	39.2	43.3	36.0
10	32.5	25.9	25.9	17.5	38.9	45.7	50.6	30.0
11	37.6	30.6	25.7	21.3	49.9	47.2	49.3	36.0
12	32.2	25.6	20.3	16.6	45.2	42.8	48.0	34.6
13	37.3	29.2	25.9	21.0	51.0	42.6	45.3	38.0
14	38.3	28.0	25.0	19.1	49.1	40.0	55.3	42.7
15	31.1	27.5	21.8	18.2	37.6	45.6	58.7	34.0
16	35.0	25.9	25.9	17.3	47.9	44.4	41.3	32.0
17	31.9	27.1	23.3	17.0	45.5	50.7	38.7	30.0
18	37.0	30.6	22.9	20.0	51.9	62.7	48.7	24.7
19	37.6	28.8	29.1	20.8	43.3	44.1	60.0	34.2
20	30.2	24.6	17.3	14.0	47.5	47.1	42.7	32.0
Ave.	35.5	28.9	25.1	20.1	47.4	46.1	47.2	34.4

Total non-protein nitrogen. The total non-protein nitrogen of the fetal whole blood varied from 26.2 to 42.5 mg. and averaged 35.5 mg. per 100 cc., while the maternal blood ranged from 23.3 to 34.4 mg. and averaged 28.9 mg. per 100 cc. The fetal blood had a uniformly higher content than the maternal blood, the average difference being 6.6 mg. per 100 cc., and the range of variation being from 2.9 to 10.3 mg. per 100 cc.

The fetal plasma showed values for the total non-protein nitrogen ranging from 17.3 to 32.9 mg., and averaging 25.1 mg. per 100 cc., whereas the maternal plasma varied from 14.0 to 27.8 and averaged 20.1. The difference was again constantly in favor of the fetus and averaged 4.0 mg. per 100 cc., with 1.3 and 8.4 mg. as the extremes.

The total non-protein nitrogen of the fetal corpuscles ranged from 32.4 to 62.7 mg. and averaged 47.4 mg., while the maternal cells ranged from 33.9 to 62.7 mg. and averaged 46.1 mg. per 100 cc. The fetal cells were higher than the maternal corpuscles in 12 cases and lower in 8 cases; in the former the range of difference was from 1.0 to 16.9 and in the latter from 1.5 to 10.8 mg. per 100 cc.

Folin and Berglund (9) give the following total N.P.N. values for normal men:

	RANGE	AVERAGE
Whole blood	27.8–39.4	32.1
Plasma	18.0–30.0	24.7
Corpuscles	37.7–55.0	43.6

while Wu (10) for his 20 normal bloods gives:

	RANGE	AVERAGE
Plasma	20.0–36.0	28.8
Corpuscles	39.0–57.0	47.8

In tabular form our results read:

	FETAL		MATERNAL	
	Range	Average	Range	Average
Whole blood..	26.2–42.5	35.5	23.3–34.4	28.9
Plasma.....	17.3–32.9	25.1	14.0–27.8	20.1
Corpuscles....	32.4–62.7	47.4	33.9–62.7	46.1

The only significant fact developing from this comparison is that the maternal whole blood and plasma are poorer in non-protein nitrogen than they are in normal non-pregnant individuals, an observation which has been repeatedly made and which may probably be attributed to a blood and plasma dilution.

Our results fail to confirm those of any previous investigator, in that they show a consistent variation of the non-protein nitrogen in favor of the fetus, but, if they are substantiated by subsequent workers, will quite definitely remove the possibility of the presence of an unknown non-protein nitrogenous substance, which varies markedly in individual cases, a hypothesis which offered the only explanation for the variations previously found, for all the known non-protein nitrogenous substances are in equal or nearly equal concentration in the fetal and maternal blood except the amino-acids which are aways higher in the fetal circulation. The fact that the average determined difference in total non-protein nitrogen between the fetal and maternal blood and plasma (6.6 and 4.0 mg. per 100 cc., respectively) is considerably greater than the observed difference between the amino-acids of the fetal and maternal whole blood and plasma (2.1 and 2.0 mg. per 100 cc. respectively) indicates that the rest-nitrogen is appreciably higher in the former. We have no knowledge as to what substances are contained in this undetermined fraction, but it may be logically assumed to be a material necessary for fetal growth.

TABLE III

Urea nitrogen

In fetal and maternal bloods

	WHOLE BLOOD		PLASMA		CORPUSCLES		CORPUSCLE VOLUME	
	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal
	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	per cent	per cent
1	8.2	7.7	6.6	7.0	10.0	9.1	48.0	35.1
2	9.9	10.4	11.6	12.3	7.9	9.0	46.6	33.3
3	12.2	12.8	14.4	16.4	9.9	5.0	48.4	31.6
4	12.3	11.2	13.3	13.4	11.2	7.3	49.0	35.5
5	7.9	7.7	7.8	8.0	8.0	7.3	40.0	38.1
6	8.3	8.1	8.7	8.7	7.6	7.1	37.0	35.4
7	15.6	15.6	18.1	18.0	12.9	10.7	47.9	32.7
8	9.5	9.7	9.9	9.9	9.0	9.5	45.3	42.0
9	10.0	10.0	10.2	10.0	9.7	10.0	43.3	36.0
10	8.7	8.1	7.9	7.9	9.5	8.7	50.6	30.0
11	11.1	11.1	11.1	11.1	11.1	11.1	49.3	36.0
12	8.4	8.8	8.8	8.8	8 1	8.8	48.0	34.6
13	11.0	11.0	11.0	11.0	11.0	11.0	45.3	38.0
14	8.7	8.7	8.7	8.9	8.7	8.4	55.3	42.7
15	7.5	7.5	7.5	7.5	7.5	7.5	58.7	34.0
16	8.4	8.5	8.5	8.5	8.5	8.5	41.3	32.0
17	8.3	8.3	8.3	8.3	8.3	8.3	38.7	30.0
18	9.4	9.4	9.4	9.4	9.4	9.4	48.7	24.7
19	11.7	11.7	11.7	11.7	11.7	11.7	60.0	34.2
20	7.7	7.7	7.7	7.7	7.7	7.7	42.7	32.0
Ave..	9.7	9.7	10.1	10.2	9.4	8.8	47.2	34.4

Urea. The fetal whole blood urea-nitrogen varied from 7.5 to 15.6 mg. and averaged 9.7 mg. per 100 cc., while the maternal values had the same range and average. In 10 of the 20 cases, the two bloods gave identical results, while among the remainder

the fetal blood was higher in 5 and the maternal blood in 5, the maximum difference being 1.1 mg. and the average difference 0.4 mg. per 100 cc., variations probably not exceeding the experimental error.

Fetal plasma showed values from 6.6 to 18.1 mg., averaging 10.1 mg. per 100 cc., whereas the maternal plasma varied from 7.0 to 18.0 mg. and averaged 10.2 per 100 cc. In 12 instances the results were identical, while in two the fetal sample was higher and in six the maternal. The maximum difference was 2.0 mg. and the average difference 0.5 mg. per 100 cc.

The calculated values for fetal corpuscles varied from 7.5 to 12.9 mg. and averaged 9.4 mg. per 100 cc., while the maternal cells showed a range from 5.0 to 11.7 mg. and an average of 8.8 mg. per 100 cc. In 8 specimens the values were identical, while in 8 the fetal cells were higher and in 4 the maternal corpuscles apparently contained more urea. Since these values are merely calculated, they tend to exaggerate any errors in the analyses of the whole blood and plasma, and should probably be given but little consideration.

Folin and Berglund present the following figures for urea-nitrogen in normal men:

	RANGE	AVERAGE
Whole blood	8.9–15.2	11.5
Plasma	9.6–17.3	12.4
Corpuscles	7.7–13.2	10.3

and Wu's values are:

	RANGE	AVERAGE
Plasma	13–23	19.3
Corpuscles	12–22	17.1

Our tabulated results show:

	FETAL		MATERNAL	
	Extremes	Average	Extremes	Average
Whole blood	7.5–15.6	9.7	7.5–15.6	9.7
Plasma	6.6–18.1	10.1	7.0–18.0	10.2
Corpuscles	7.5–12.9	9.4	5.0–11.7	8.8

Choosing for comparison the values given by Folin and Berglund, it will be seen that our figures average slightly lower. This accords with the results of previous investigations upon the blood during pregnancy, as does the observation that generally the plasma contains slightly more urea than the corpuscles.

Slemons and Morriss (6), Morel and Mouriquand (3), and Howe and Givens (7) have obtained similar results on whole blood, but we have found no record of previous work on the plasma, although the great diffusibility of urea made it very probable that its concentration would be the same in the fluid portions of the two bloods.

Uric acid. Fetal whole blood contained from 1.0 to 4.7 mg. of uric acid (average 2.6 mg.), while the maternal whole blood values ranged from 1.3 to 3.8 mg. and averaged 2.5 mg. per 100 cc. In 7 instances identical values were obtained, in 10 the fetal specimen was slightly higher and in 3 the maternal blood contained slightly more uric acid. The differences varied from 0.1 to 0.9 mg. per 100 cc. and averaged slightly less than 0.4 mg.

The uric acid values for fetal plasma ranged from 1.2 to 5.7 mg. and averaged 2.8 mg. per 100 cc., whereas the maternal plasmas varied from 1.2 to

5.2 mg. and averaged 2.6 mg. per 100 cc. In 11 cases, the two specimens agreed absolutely, while in the remaining 9 the fetal plasma was higher. In no instance did the maternal plasma

TABLE IV

Uric acid

In fetal and maternal bloods

	WHOLE BLOOD		PLASMA		CORPUSCLES		CORPUSCLE VOLUME	
	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	Maternal
	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	mg. per 100 cc.	per cent	per cent
1	2.2	1.9	2.5	2.5	1.8	0.8	48.0	35.1
2	2.2	2.8	2.8	2.6	1.5	3.3	46.6	33.3
3	2.6	2.2	3.0	2.0	2.2	2.5	48.4	31.6
4	3.2	3.1	3.2	3.2	3.3	2.8	49.0	35.5
5	3.0	3.0	3.2	3.2	2.7	2.9	40.0	38.1
6	2.8	2.7	2.8	2.8	2.9	2.5	37.0	35.4
7	3.8	3.6	2.7	2.7	5.0	5.5	47.9	32.7
8	3.3	2.8	3.3	3.2	3.1	2.1	45.3	42.0
9	4.7	3.8	5.7	5.2	3.4	1.3	43.3	36.0
10	1.3	1.3	1.2	1.2	1.4	1.6	50.6	30.0
11	2.9	3.1	3.1	3.1	2.6	3.1	49.3	36.0
12	1.0	1.3	1.9	1.1	2.0	1.7	48.0	34.6
13	1.5	1.5	1.5	1.5	1.5	1.5	45.3	38.0
14	2.6	2.2	2.6	2.2	2.6	2.2	55.3	42.7
15	2.3	2.3	2.3	2.3	2.3	2.3	58.7	34.0
16	1.5	1.3	1.5	1.3	1.5	1.3	41.3	32.0
17	1.5	1.5	1.5	1.5	1.5	1.5	38.7	30.0
18	2.5	2.5	2.7	2.5	2.3	2.5	48.7	24.7
19	3.7	3.4	3.7	3.6	3.8	2.9	60.0	34.2
20	3.8	3.8	4.6	4.6	2.8	1.9	42.7	32.0
Ave..	2.6	2.5	2.8	2.6	2.5	2.3	47.2	34.4

contain more uric acid than the fetal. The established difference varied from 0.1 to 1.0 mg. and averaged 0.4 mg. per 100 cc.

The fetal corpuscles were calculated to contain from 1.4 to 5.0 mg. per 100 cc. and averaged 2.5 mg., while the maternal blood cells ranged from 0.8 to 5.5 mg. and averaged 2.3 mg. The two samples agreed in only 4 instances, whereas in 10 cases the fetal specimen was higher and in 6 the maternal. The difference varied from 0.2 to 2.1 mg. and averaged 0.7 mg., the probable errors in the determined values for whole blood and plasma being again exaggerated.

Wu's normal values are:

	EXTREMES	AVERAGE
Plasma	2.3–5.7	3.9
Corpuscles	1.2–3.8	1.9

while our tabulated results are as follows:

	FETAL		MATERNAL	
	Extremes	Average	Extremes	Average
Whole blood	1.0–4.7	2.6	1.3–3.8	2.5
Plasma	1.2–5.7	2.8	1.2–5.2	2.6
Corpuscles	1.4–5.0	2.5	0.8–5.5	2.3

Our values for plasma tend to be lower than those of Wu but the corpuscular content is appreciably higher and more nearly equally to the plasma values. We have noticed in other previous work that the corpuscles of pregnant women apparently contain more uric acid than normal, but have no explanation to offer for this fact.

Our results confirm those of Kingsburg and Sedgwick (14), Slemons and Bogert (15), and Howe and Givens (7), that the uric acid does not show any consistent variations between fetal and maternal whole blood, and advance the new fact that equivalent values are likewise present in the plasma and corpuscles. We also noted, as did Slemons and Bogert, that occasionally at the end of labor the

uric acid of the whole blood is slightly above normal.

Hematocrit. The corpuscle volume percentage, as determined by the hematocrit readings, varied constantly in favor of the fetus, the difference ranging from 1.6 to 25.8 volume per cent with an average of 12.8. The fetal cell volume ranged from 37.0 to 60.0 and averaged 47.2 volume per cent, whereas the maternal cell volume ranged from 24.7 to 42.7 and

TABLE V

Clinical data

Number	Age	Number of previous children	Duration of labor in hours	Anesthetic	Blood pressure	Amount of blood lost	Remarks	Fetus		
								Sex	Weight	Condition at birth
						cc.			grams	
1	16	0	34–10/60	Ethylene-ether	125/80	100	Spontaneous prolonged labor	M.	2,475	Good
2	32	2	4	Ethylene-ether	105/70	80	Spontaneous	F.	3,375	Good
3	28	0	7–35/60	Ethylene-ether	100/65	100	Low forceps	M.	3,520	Excellent
4	29	1	2–30/60	Ethylene		200	Spontaneous	F.	3,410	Excellent
5	30	3	5– 5/60	Ethylene-ether		200	Spontaneous	M.	3,265	Good
6	24	0	14	Ethylene-ether	90/55	350	Spontaneous	F.	3,525	Good
7	34	1	9– 7/60	Ethylene-ether	125/75	75	Spontaneous	M.	3,160	Good
8	39	1	6–14/60	Ethylene-ether	135/90	300	Spontaneous	M.	3,155	Good
9	36	2	6–20/60	Ethylene-ether	130/90	400	Spontaneous	F.	4,075	Excellent
10	29	4	1–20/60	Ethylene-ether	110/65	550	Spontaneous	M.	3,950	Excellent
11	21	0	35–40/60	Ethylene-ether	100/60	350	Spontaneous prolonged labor	F.	2,650	Excellent
12	22	0	8–55/60	Ethylene-ether		50	Breech extraction	F.	3,080	Fair
13	32	0	2–50/60	Nitrous oxide-ether	155/105	500	Spontaneous precipitate labor	F.	3,340	Excellent
14	36	5	3–31/60	Nitrous oxide-ether	105/65	400	Spontaneous	M.	4,405	Excellent
15	28	2	6–25/60	Nitrous oxide-ether	100/70	300	Spontaneous	F.	3,280	Excellent
16	24	1	2–40/60	Nitrous oxide-ether	110/65	275	Spontaneous precipitate labor	F.	3,075	Excellent
17	32	3	5–20/60	Nitrous oxide-ether	120/80	400	Spontaneous	F.	4,040	Excellent
18	32	1	9–20/60	Nitrous oxide-ether	115/80	415	Spontaneous	M.	3,515	Excellent
19	30	1	3–30/60	Nitrous oxide-ether	125/85	750	Spontaneous	M.	3,815	Excellent
20	30	0	5–10/60	Ethylene	110/70	550	Spontaneous	F.	3,260	Excellent

averaged 34.4 volume per cent. These findings agree with those of other investigators (16) that the fetal blood contains a greater percentage of cells than does the maternal.

Clinical data. We have attempted to study only normal individuals and the clinical data presented in Table V indicate that in no mother was there any particular sign of disease.

We have been unable to correlate the age or parity of the mother with any significant changes in the blood relationship, nor do any of the other factors charted seem to affect the variations recorded in our experiments.

SUMMARY

The fetal and maternal whole blood and plasma from 20 normal patients at the term of delivery were analyzed for amino-acids, total non-protein nitrogen, urea and uric acid. It was found that the amino-acid nitrogen is constantly higher in the fetal whole blood and plasma than in the maternal samples, thus confirming the investigations of Morse on plasma and of Bock on whole blood. The amino-acids, which are very probably necessary for the synthesis of proteins in the fetal organism, are thus seen to behave as do the other constituents, which are essential for growth (calcium and inorganic phosphates). It would seem that the preferment thus shown for the developing product of conception must be the result of the working of a definite physico-chemical law which acts to insure the fetus an overabundance of the essential "building stones." Simple diffusion does not explain the facts, and "selective absorption" is too indefinite to be of any value.

Largely by reason of this constant difference in the amino-acids and the established uniformity of the other non-protein nitrogenous constituents in the circulations, the fetal whole blood and plasma were found to contain constantly more total non-protein nitrogen than the maternal specimens. Such constant results have not been obtained by previous investigators, who came to the conclusion that these substances pass the placenta probably by simple diffusion. The urea and uric acid in the two bloods tend to be equal and in all probability the slight differences detected at times may have been due to analytical errors. No definite relationship could be detected between the variations detected in the individual substances and any facts obtained from clinical study of the patients.

CONCLUSIONS

1. The amino-acids are uniformly higher in normal fetal whole blood and plasma than in the maternal.

2. The total non-protein nitrogen is likewise always in higher concentration in the fetal whole blood and plasma.

3. Urea is usually found in equal concentrations in the two bloods.

4. Uric acid is usually found in equal concentrations in the two circulations, but, if there is a difference, it is generally higher in the fetal blood.

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NOTE

Since this communication was sent to the press, the report of a similar piece of work by Karl Hellmuth has appeared in the Archiv für Gynäkologie, 1924, CXXIII, 57–87 (Beiträge zur Biologie des Neugeborenen. I. Mitteilung: Untersuchungen über die Verteilung des Nichtprotein-Stickstoffs und seiner Hauptkomponenten (des Harnstoff—N, Aminosäure—N, der Harnsäure, des präformierten und Gesamtkreatinins im mütterlichen und kindlichen Blut unter normalen und pathologischen Verhältnissen). This author examined the whole blood of mother and child and found very much the same variations as we have here reported. The total non-protein nitrogen was not always higher in the fetal circulation, although this was usually the case, the urea and uric acid were usually the same, while the amino-acid nitrogen was uniformly higher in the fetal blood, the average difference being 2.2 mg. per 100 cc.

The Histo-pathology of Post-encephalitic Parkinson's Syndrome[1]

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In a recent communication (1) I reported the histo-pathological findings in 11 cases of Parkinson's syndrome following Epidemic Encephalitis. In that publication the literature was reviewed and a detailed account of the clinical records and the pathological findings of the 11 cases was given.[2] In the present paper I wish briefly to summarize the findings in those cases and add an additional case, observed during life in this Clinic and studied in its Neurological Laboratory. We felt that our group of cases was of more than ordinary interest because of the relatively large number at our disposal. With the exception of our material there are probably less than twenty cases reported and these have been available only in groups of one or two.

In general, we were able to confirm the findings of most of the other investigators (1) that the process, is a very wide-spread one which involves practically every part of the central nervous system—cortex, basal ganglia, midbrain, cerebellum, medulla and cord, with especial localization in the basal ganglia and midbrain tegmental structures; and (2) that the process affects the parenchyma primarily and the cell changes are for the most part of the severe chronic degenerative sort with cell shrinkage, and sclerosis, on the one hand, and cell dissolution, on the other, with neuronophagia. Along with this constant picture of chronic degeneration one also finds more acute alterations with swelling and axonal alteration. The nerve fibers show all stages from acute swelling to complete resorption of large areas of white matter. The process may be a loss of myelin in a somewhat discontinuous fashion or a more radical loss resulting in a cribriform appearance. This formation of holes in the tissues may or may not implicate blood vessels. The glia, on the whole, shows surprisingly little activity. There is to be found some increase about the blood vessels with the production of glia fibers, but in general the glial increase is mainly cellular and manifests itself diffusely, or in the formation of small glia nodules.

The mesenchymal elements show relatively little change. There is some slight thickening and the appearance

[1] Read before the Johns Hopkins Medical Society, February 11, 1925.

[2] These cases were studied in the laboratory of Professor Marburg of the Neurological Institute of the University of Vienna. I wish to acknowledge my indebtedness to Professor Marburg and also to Dr. E. Pollack for their kindly and helpful assistance.

of calcareous deposits in the walls of the blood vessels, which is not to be accounted for by the age of the patients. The pia shows signs of irritation with minimal evidences of meningitis.

A most striking feature is the universal finding in our cases of persistent signs of acute and subacute inflammatory reaction even after months or years of the disease. In every case, if one searches diligently enough, one finds definite areas of round-cell infiltration with plasma cells and lymphocytes. In some cases one might have to search many sections before finding it, but it was always present. Sometimes, when the infiltration was massive, one could find an occasional polymorphonuclear leucocyte as proof of the relative acuteness of the process. The infiltration was almost exclusively perivascular. No part of the nervous system escaped this inflammatory process from cortex to cord.

In addition to the above we found a striking fatty change in the cells, which varies from a mere lipoid increase to a fatty dystrophy. Even taking into account the fact that the patients as a result of a prolonged illness associated with difficulties in deglutition were profoundly marantic, the fatty changes seemed out of proportion to the amount one could expect from this cause.

We were naturally interested to learn whether the present group of cases would throw any light upon the question of central nervous system localization in the Parkinson syndromes, especially since there has been so much conflicting evidence presented by investigators who have studied the so-called extrapyramidal syndromes. It is clear to us that no process which involves the central nervous system in so wide-spread a fashion can give any conclusive evidence on this question of localization, and we are prepared to present our evidence only in the most conservative fashion. The schools of neuropathologists who have studied the akinetic hypertonicity syndrome of Parkinson have dealt for the most part with senile brains. These investigators, led by the Vogts and Ramsey Hunt, have been impressed with the lesions in the globus pallidus and large cells of the caudate and putamen. The French School of Pierre Marie and his pupil Tretiakoff, on the other hand, in their study of the syndrome in senile cases and in post-encephalitic cases, have postulated the most important lesion in the substantia nigra. There has been practical agreement by all other authors, that in the cases of encephalitic Parkinsonismus described up to date the substantia nigra is the most profoundly involved. There is, of course, no necessity for believing that both sides may not be correct and that the localization in the two diseases is not different. The fact that, whatever the pathways involved in the extra-pyramidal syndromes, they are constituted by a number of links, would make it easy to understand that an interruption at one of several places might result in the same final outcome. At any rate, in all of our cases,[3] including the one reported in this paper, the most profound and constant degeneration was found in the substantia nigra. The degeneration in the substantia

[3] In one case neither the substantia nigra nor pallidum were available for examination.

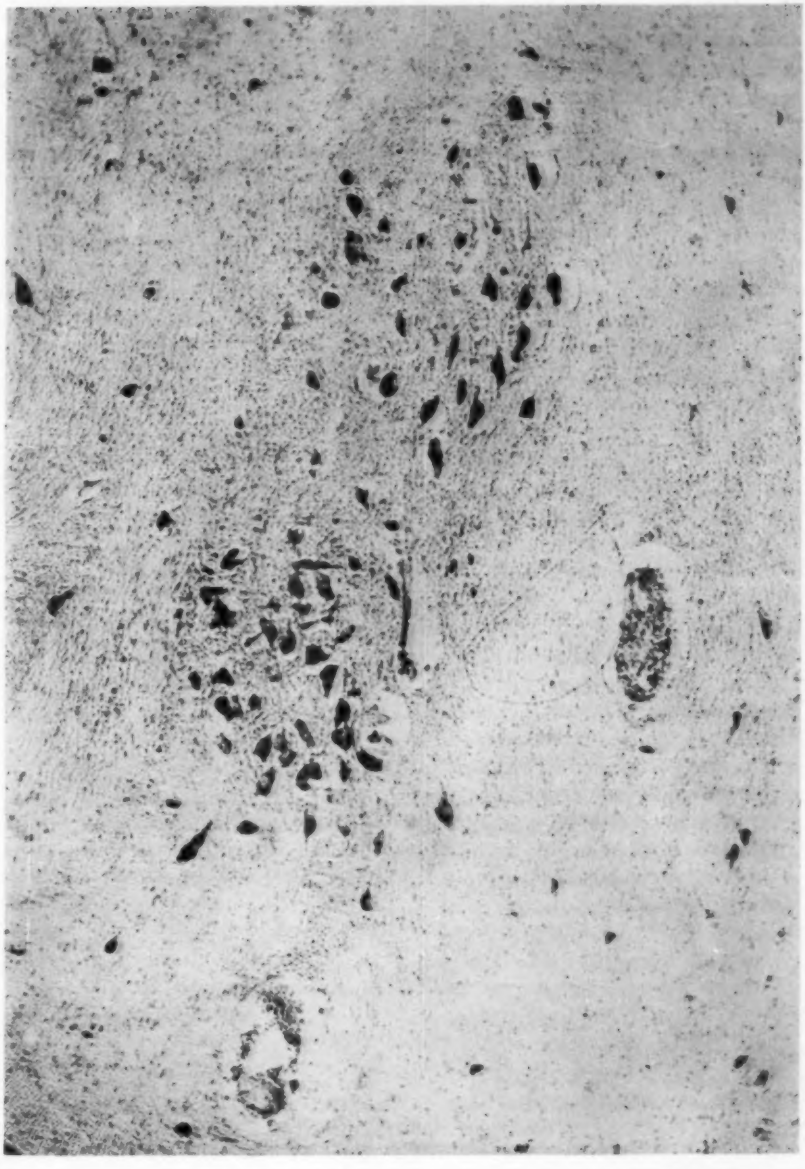

FIG. 1. THE NORMAL CELL CONFIGURATION OF THE SUBSTANTIA NIGRA FROM AN ACUTE CASE OF EPIDEMIC ENCEPHALITIS

The magnification Figs. 1 to 4 is × 125

nigra is manifested by the disappearance of a very great number of the large pigmented cells, destruction of many of the fibers and an increase of glia elements with pigment inclusions and pigment masses lying free in the tissues. Further, cases were found in which the pallidum was practically intact. Next in order of severity of involvement was the striatum (putamen and caudatus) and then, thirdly, pallidum, cerebral cortex, the midbrain tegmental structures (Darkschewitsch, nucleus interstitialis, and nucleus ruber); then the cerebellum with the dentate, the nuclei of the medulla (dorsal vagus nucleus and ambiguus), and, finally, the cord.

The case to be reported in this paper is particularly interesting because (1) the pathological changes except in the substantia nigra were almost negligible; (2) the evidences of inflammation were so slight that only after repeated searches through many blocks of tissue could it be found and then mainly in the region of the substantia nigra. The case reported is the twelfth of my series. The clinical record is followed by the histopathological report of the brain and cord. Grossly, no abnormalities were observed in the brain.

CLINICAL RECORD

Case XII. 3017.

C. A. B. Aged 36. Married. Clerk. Admitted to the Phipps Clinic March 12, 1920, and died June 17, 1921, after a 14 months' illness.

The patient's *personal history* is negative except for slight alcoholic excess.

The *present illness* which brought him to the hospital began on March 12, 1920, with dizziness, low fever, pain in his elbow, overtalkativeness and euphoria. A week later he became blue, listless, and seclusive. On the 20th he had generalized tremor, became sleepless, and very restless. This continued and on the 29th, in addition, his speech became thick, he had difficulty in swallowing, and developed diplopia.

On admission on the 12th of March, the patient showed a speech defect, and deep depression with fear of death. *Neurologic examination* revealed: (1) left pupil larger than right but both reacting promptly to light and accommodation, although the movements were slight. Negative eyegrounds; (2) weakness of the jaw muscles; (3) dysphagia (necessitating tube-feeding), and a coarse tremor of the tongue; (4) subjective numbness and pain in the extremities; (5) hyperactive but equal deep and superficial reflexes; (6) a mask-like facies with coarse tremor of the extremities; (7) general weakness. He held positions as if cataleptic. He looked sick and had a temperature of 100.6° with 12,600 leucocytes. His condition remained stationary until the 11th of April, 1920, when he developed a right facial weakness, and a cardio-respiratory attack with a pulse of 120 and a respiration rate of 32 with marked sweating. At the same time his urine was heavy with albumin and contained many casts. The following day he became comatose, with jerking of the eyeballs and of all of the musculature of the extremities. Lumbar puncture showed 40 cells, globulin ++, and a luetic zone gold sol. curve. These spinal fluid findings remained constant until the end except for a decrease in cells just before death. He improved gradually from this attack but on April 26 showed rigidity of the neck with a positive Kernig on the right. On May 12, his fever was higher, with the deep reflexes more active on the left, and pupils fixed to light.

From this time on there was no essential change in his condition except that the pyrexial periods with an increased respiratory rate came in irregular periods and there was a general continuous increase in rigidity. The rigidity assumed the Parkinsonian attitude with flexion of the arms and tremor of the hands but no pill-rolling movements. The patient could not move in bed. By May bed-sores had developed and in spite of the most careful

nursing care they could not be healed. The fever ranged from 99° to 103° and was practically present during the whole illness. A mild cystitis was present for the last 4 months of the illness. He had incontinence of the urine practically the entire time. He died on June 17, 1921, of bronchopneumonia.

HISTOLOGICAL FINDINGS IN THE CENTRAL NERVOUS SYSTEM

Right putamen and caudatus: The number of small and large cells is clearly not diminished. Many of the large cells are practically normal, but there are also cells that show severe alterations, of various stages of chronicity. One can observe cells which are mere shadows, others in which the cell body is honey-combed, and still others in which there is definite evidence of cell shrinkage with clumping of the Nissl substance. In some cells the nucleus has been pushed to one side and practically extruded from the cell. Again, one sees definite evidence of dissolution of cell walls. The small cells are in part normal but one also sees nuclear swelling, and a moth-eaten appearance of the cell borders. Thorough search through the nucleus fails to reveal any evidence of inflammatory change. On the other hand, no increase of glia can be detected. Many of the vessel spaces are widely dilated and the surrounding tissue suggests an increase of glia fibers.

In the *right globus pallidus* the number of large cells is normal, and, in contrast to those of the putamen, are in a much better state of preservation. The Nissl substance is not distinctly to be made out, but one must take into account the fact that the material has been in formalin for three years; that the autopsy was performed 18 hours after death; that the patient had a prolonged period of fever, and was marantic for many months. Here, again, in the putamen there is no evidence of inflammatory reaction.

In the *left putamen* one gets definitely the impression that the large cells are reduced in number, but that on the whole they are less severely implicated. It is true that cell shadows are present and that the Nissl substance is by no means clear, but, by and large, the remaining cells are well preserved. The small cells also are much better preserved than on the right and show no reduction in number. Much more striking than the cell changes noted above is the condition to be found in both *left and right substantia nigra*. Here one finds that whole islands of the pigmented cells have been completely wiped out, all that remains of them being represented by a group of pigment clumps and a certain number of glia cells filled with pigment. The remaining pigmented cells show various stages of degeneration—from shrinkage to actual dissolution of the cell borders. The vessel spaces in this region do not show any unusual dilatation, nor is there any marked glial overgrowth. In some of the larger vessels in the substantia nigra there is a very slight round-cell infiltration. Apparently the number of cells has been reduced at least two-thirds. On the other hand, the cells of the *corpus subthalamicum* are very well preserved and are to be regarded as normal.

In the *left globus pallidus* the number of cells is normal and the cell structure seems to be intact. Nowhere is there any evidence of glia increase, neuronophagia, or small-cell infiltration.

In the *medulla oblongata* one finds the olives relatively intact with surprisingly little pigment. The Nissl substance is not well marked but no less so than in the remainder of the brain.

The cells of the *nucleus ambiguus* show alterations which, however, are no more than would be expected from fever, *i.e.*, partial disappearance of the Nissl substance, swelling, and beading of the nuclear membrane.

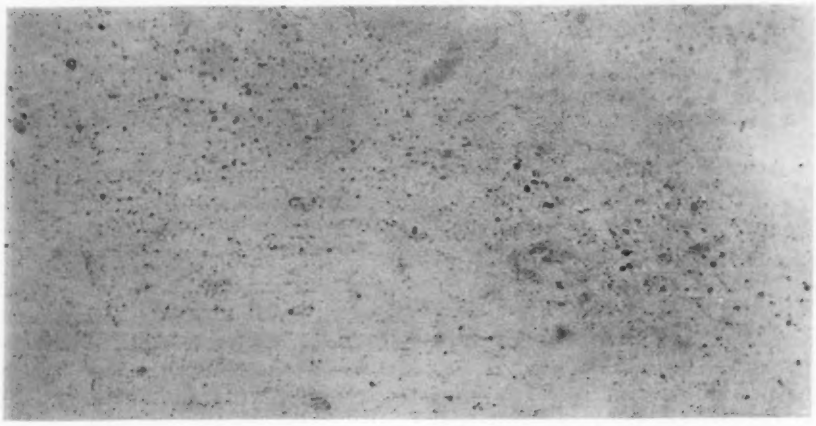

FIG. 2. DEGENERATED RIGHT SUBSTANTIA NIGRA FROM THE CASE REPORTED (CASE XII)

Practically no cells remain. Only pigment clumps and glia cells with pigment are visible.

FIG. 3. DEGENERATED LEFT SUBSTANTIA NIGRA FROM THE CASE REPORTED (CASE XII)

Nigral cells degenerated. Only vestiges of pigment remain

The large motor cells of the reticular substance and the dorsal vagus nucleus show somewhat more severe changes of the same order. Nowhere in the medulla is there any evidence of round-cell infiltration.

In the *spinal cord* the anterior horn cells show the same febrile alteration, but are otherwise not strikingly abnormal.

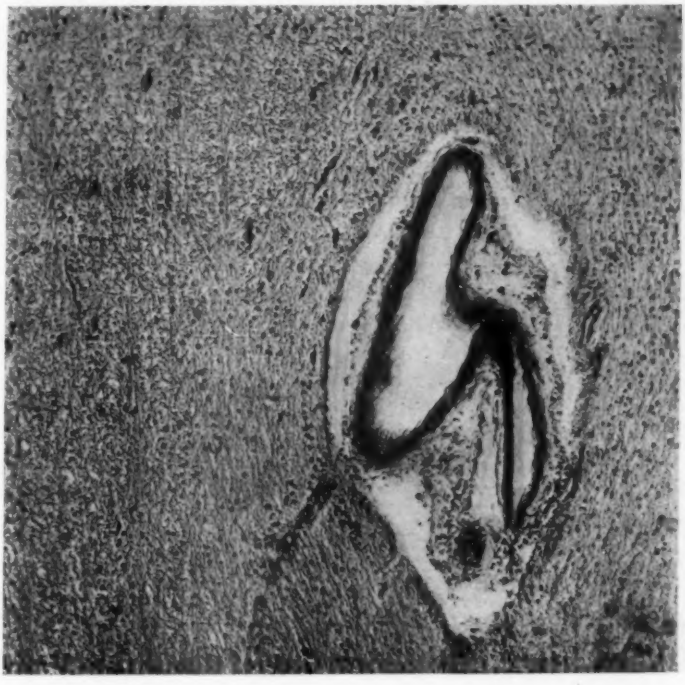

Fig. 4. Round-cell Infiltration in a Vessel of the Left Substantia Nigra

In the left *precentral cortex* one can observe small focal areas of cell loss. There is definite evidence of cell damage especially in the larger pyramidals. Undoubtedly a large part of the alterations here, however, are due to changes due to fever and long-standing inanition. In the majority of these cells the nuclei are excentrically placed, there is a complete loss of Nissl substance, and many cells are completely homogeneous without any remains of cell structure. Here, again, in the cortex no evidence of round-cell infiltration is apparent. In a section from the left frontal cortex, one finds normal architectonic patterns, but the cells are deeply stained, shrunken, and there are no Nissl bodies. If it were not for the doubtful fixation of the tissues one would be inclined to regard many of the cells as sclerotic.

Here as elsewhere in the cortex there is no evidence of inflammatory reaction, and the cells are on the whole well preserved.

In *summary*, therefore, one can say that, except for the changes due to fever and poor fixation, one finds: (1) evidence of moderate cell degeneration in the corpus striatum with an intact globus pallidus; (2) a severe and profound destruction of the substantia nigra; (3) focal areas of loss in the cerebral cortex in both cells and fibers; (4) evidence of definite but very slight inflammatory changes; (5) very little glia increase.

SUMMARY

In a summary of 11 cases previously reported and an additional new case the lesions of post-encephalitic Parkinson's syndrome were found to consist in:

(1) An essentially chronic degenerative parenchymatous process with constant persistent evidences of inflammatory reaction.

(2) Wide-spread distribution of the lesions in every part of the central nervous system.

(3) The region of maximal and constant involvement is the substantia nigra.

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Chemical Studies on the Toxemias of Pregnancy

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THE finding of a completely satisfactory method for differentiation between the various types of late toxemias of pregnancy and the discovery of the etiology of eclampsia are still among the leading unsolved problems in the field of obstetrics. The development of micro-chemical methods has made it possible to attack these problems from a purely chemical side and has resulted in some valuable and pertinent information.

Calcium deprivation of the mother resulting from the drain on the maternal organism by the product of conception has furnished some investigators a field for speculation as to the etiology of eclampsia. Plass and Bogert have recently reviewed this subject and have drawn attention to the fact that not only is there great divergence of opinion with regard to the normal variations in the calcium content of the blood during gestation, but also that the series of cases reported by most workers have been far too small to allow general deductions. This criticism seems well warranted.

Plass and Bogert then reported the calcium and magnesium values in a large series of cases—205 normal non-pregnant, pregnant, parturient and puerperal women—and found a definite and consistent tendency toward a lowering of serum calcium during gestation. Their average values for serum calcium were lower in normal pregnant women than in normal non-pregnant women. In women with toxemias of pregnancy, they noted no marked change in calcium from the normal pregnant women. Their magnesium determinations revealed very slight, if any, changes from the normal.

More recently, Denis and King reported values for the inorganic constituents of blood in normal and abnormal pregnancy. These authors found that in normal pregnancy the blood calcium falls within the limits for normal non-pregnant women. Furthermore, their analyses seem to indicate that there is no appreciable change in the calcium, magnesium, sodium, potassium, chloride, phosphorus and sulphur contents of the blood in toxemia cases as compared with normal pregnancy. Although opinions differ as to whether there are any marked variations in the calcium content of the blood during pregnancy, there seems, however, to be an agreement that the late toxemias of pregnancy are not accompanied by any decrease in blood calcium, as has been supposed by some authors.

During the past three years we have analyzed the blood and urine of a large number of normal non-pregnant, normal pregnant and abnormal pregnant women. The pregnant women were all close to or at term. A few determinations were also done on patients during the puerperium.

TABLE I

Normal non-pregnant

CASE NUMBER	B.P.	Oedema	ALBUMIN	T.N.%	U.N.%	NH_3N%	N.P.N.	B.U.N.	Ur.Ac.	SUGAR	CO_2	NaCl	Na	K	Ca	Mg	P	Fe
16	110/75	None	None	0.42	81.0	2.1	30.9	16.62	2.6	103	56.0	500	312	20.0	9.5	2.4	2.4	50.8
18	115/80	None	None	0.31	85.6	2.4	33.0	19.01	3.1	102	58.2	514	303	20.2	9.8	2.6	2.8	49.7
19	108/68	None	None	0.45	76.0	4.0	27.3	8.43	3.0	90	57.6	521	330	19.0	10.2	2.8	2.6	55.5
43	116/70	None	None	0.88	80.0	4.0	29.8	8.41	2.9	100	58.1	571	337	19.2	10.0	3.0	2.4	
44	117/75	None	None	0.71	82.2	3.0	31.0	14.05	3.4	98	52.3	528	311	21.6	9.4	2.7	2.4	
47	118/63	None	None	1.167	71.0	4.9	27.7	13.55	3.1	137	55.7	534	314	21.4	10.6	3.0	3.1	50.0

TABLE II

Normal pregnancy

CASE NUMBER	B.P.	OEDEMA	ALBUMIN	T.N.%	U.N.%	NH_3N%	N.P.N.	B.U.N.	Ur.Ac.	SUGAR	CO_2	NaCl	Na	K	Ca	Mg	P	Fe
1	120/80	None	None	0.61	80.0	2.5	31.4	13.01	2.4	103	46.2	536	313	21.4	9.8	2.5	2.3	35.2
5		None	None	0.56	73.2	3.6	29.1	14.01	2.7	100	45.7	485	330	18.5	9.3	2.2	2.3	
13	100/70	None	None	0.59	78.0	4.5	28.1	11.67	2.8	102	43.7	471	335	23.9	8.9	2.4	3.0	48.2
42	115/80	None	None	0.90	79.1	2.1	27.3	12.50	3.2	91	40.0	485	306	21.5	9.8	2.3	2.6	
58	120/85	None	None	0.82	77.1	2.4	27.4	12.12	2.7	95	48.1	494	340	20.5	10.2	2.1	3.1	

METHODS

Urine: All analyses were made on 24-hour specimens. For the determination of total nitrogen we used the procedure outlined by Folin; for urea nitrogen that of van Slyke and Cullen; and for ammonia-nitrogen we followed the directions of Folin.

Blood: Blood specimens were taken from an arm-vein, before breakfast, except in cases of eclampsia. In these we have endeavored to obtain samples immediately after a convulsion. Lithium citrate was used as an anticoagulant. The non-protein nitrogen and sugar were determined by the Folin-Wu methods, the blood urea-nitrogen by the Van Slyke-Cullen modification of the Marshall method,

the chlorides by the Whitehorn method, and the CO_2-combining power according to Van Slyke's technique. Whole blood was used in the above determinations, for reasons already set forth by one of us. As soon as the blood was obtained from the patient, a Folin-Wu filtrate was immediately made. This is especially important if sugar determinations are to be made.

For the determination of inorganic elements we used blood plasma, except in the case of iron, where whole blood was employed. For potassium, magnesium and phosphorus, we followed the modifications of Briggs for the colorimetric methods; for sodium the Kramer and Tisdall procedure, as well as the Kramer and Gittelman iodometric method; for calcium Briggs' modification of the Kramer method, and for iron the method of Wong. All determinations were done in duplicate or triplicate, or until satisfactory checks were obtained.

TABLES

In the accompanying tables the total nitrogen (T. N.) is expressed as a percentage of the total 24-hour urine, to indicate the extent of dilution; the urea nitrogen (U. N.) and the ammonia -nitrogen (NH_3-N.) are expressed as percentages of the total nitrogen. The non-protein nitrogen (N.P.N.) the blood urea-nitrogen (B.U.N.), the uric acid (Ur.Ac) the sugar and the chlorides of the blood are given in milligrams per 100 cc. of blood; the CO_2-combining power of the blood is expressed in volumes per cent. All the inorganic elements are written in milligrams per 100 cc. of blood. Phosphorus represents PO_4 as P.

In connection with each case, information is given concerning the blood pressure, oedema and albumin, so that one may gain an idea of the severity of the toxemia. In the blood pressure column we have noted the highest systolic and diastolic pressures, as well as the discharge systolic and diastolic pressures in the order mentioned. The same has been done in the albumin column, where the first figure usually represents the highest amount of albumin found, and the second the amount noted on the date of discharge, usually three weeks after delivery.

In the ratio tables the first figure represents the blood urea nitrogen expressed as a percentage of the non-protein nitrogen, $\frac{\text{B. U. N.}}{\text{N. P. N.}}$. The ratio $\frac{\text{B. U. N.}}{\text{U. N. \%}}$ is the blood urea nitrogen expressed as percentage of the urea nitrogen percentage in the urine, and is the ratio designated as "x" in the publication of Stander, Duncan and Moses.

RESULTS

Normal pregnancy. A study of Tables I and II, as well as of the averages reported in Table XI, clearly indicates that normal pregnancy is accompanied by very slight changes except in the CO_2-combining power. In general there is a tendency toward lower values in the non-protein nitrogen and the blood urea nitrogen, as was previously shown by one of us. The decrease in the CO_2-combining power is well known and gives evidence of an existence of the "acidosis of pregnancy."

At present we are using a purely arbitrary method for differentiation between the various types of late

TABLE III

Nephritic tozemia

CASE NUMBER	B.P.	OEDEMA	ALBUMIN	T.N.%	U.N.%	NH₃N%	N.P.N.	B.U.N.	Ur.Ac.	SUGAR	CO_2	NaCl	Na	K	Ca	Mg	P	Fe
2	180–110 / 110–70	Marked	3.75–trace	0.45	73.3	3.5	28.4	15.17	3.1		50.3	400			10.6	2.8		
2				0.50	67.5	2.7	33.3				50.4		349	31.6	7.9	2.6		
2													325	31.6	8.0	2.5		
21	220–160 / 130–115	Marked	21–0.75	0.64	67.9	8.0	34.7	14.01	4.9		45.3	471	336	24.5	10.6	3.0		
21				0.38	88.0	2.7	34.9	16.35	3.7		44.9	493	319	22.2		2.4	2.9	43.0
32	190–115 / 110–60	Marked	16–0.3	0.26	65.7	4.8	33.0	14.48	5.9		25.2	505	347	21.4		3.6	2.5	47.8
32				0.35	89.1	6.5	33.3	21.01	3.5		46.6	534	317	24.6	10.7	2.8	2.3	30.0
6	180–150 / 110–95	Slight	3–0.25	0.28	67.5	3.8	18.6	9.34	4.9		44.3	511	340	19.5	9.1	2.6	2.4	
7	220–160 / 130–100	Slight	8–trace	0.34	61.1	6.0	23.6	10.27	4.0		42.4	478	321	22.2	9.0			
25	150–125 / 90–95	None	3–0.75	0.19	89.5	7.6	37.0	11.40	4.4		38.5	521	393	23.0	10.5	2.4	2.5	35.0
25				0.26	70.8	6.5	36.6	13.54	4.7		39.5	554	390	32.0	10.4	2.9		40.0
34	210–130 / 130–80	Slight	1.5–0.2	0.53	81.2	4.7	32.6	24.29	4.7		47.3	576	277	26.6	12.9	3.0	2.6	50.2
46	220–180 / 135–115	Marked	3–trace	0.71	80.4	7.7	23.8	9.81	3.6	105	39.6	478	319	20.0	11.7	2.7	3.1	53.0
52	180–120 / 120–80	None	Trace–0	0.25	71.4	5.6	23.0	5.07	3.6	95	45.3	544	313	22.9	11.0	2.9	3.3	50.0
53	180–120 / 120–80	None	1–0	0.41	75.4	3.2	27.3	12.64	4.4	87	51.1	485	318	18.7	12.7	2.9	3.4	55.5
57	220–150 / 150–110	None	2–trace	0.29	77.4	7.2	26.0	8.27	3.3	124	44.3	522	301	25.1	9.6	2.5	3.7	55.0

TABLE IV

Pre-eclamptic toxemia

CASE NUMBER	B.P.	OEDEMA	ALBUMIN	T.N.%	U.N.%	NH_3N%	N.P.N.	B.U.N.	Uric Ac.	SUGAR	CO_2	NaCl	Na	K	Ca	Mg	P	Fe
11	80–115 / 110–75	Slight	1–0				27.7				42.4		289	25.7			2.1	
30	190–100 / 120–80	None	0.8–trace	0.32	78.1	8.0	28.2	10.27	3.3		44.9	539	375	23.5	10.8	2.9	2.5	47.4
30				0.37	68.8	7.9							313					
3	175–115 / 110–80	Moderate	1.75–trace	1.30		3.4	27.7		7.0	100	56.4	511	310	30.6	8.5	2.6	2.4	
48	150–145 / 85–75	Slight	1.5–0.4	0.26	70.0	6.1	23.0	8.11	4.2	98	48.1		299	16.0	11.4	2.8	2.1	21.7

TABLE V

Eclampsia

CASE NUMBER	B.P.	OEDEMA	ALBUMIN	T.N.%	U.N.%	NH_3N%	N.P.N.	B.U.N.	Ur.Ac.	SUGAR	CO_2	NaCl	Na	K	Ca	Mg	P	Fe
4											46.2		372	35.5	10.0		3.5	33.3
8	240–110 / 120–0	None	4–0.5	0.66	54.4	8.3	26.3	9.34	11.9		40.3	544	401	30.6	9.1	3.1	3.6	
10	260–110 / 130–70	Marked	15–0				28.5				50.7			24.4	10.7		2.9	
12													355	40.3	9.9		3.3	
20	190–105 / 120–75	Slight	10–0	0.44	77.0	6.7	32.6	22.42	6.2		20.2	495	305	25.0	11.3	2.3	3.3	66.6
20													305	26.7	10.6	2.5	3.7	45.5
20													318			2.9		
23	200–130 / 100–75	Marked	1.75–0.1	0.30	62.0	8.3	32.6	12.14	5.8		43.8	508	360	16.0	10.7	4.2	2.5	40.0
23				0.32	75.0	5.2	31.6	11.68	3.8		48.5	498	359	25.1	11.1			
24	175–110 / 108–80	Slight	1.75–trace	0.44	66.8	4.4	28.0	15.41	9.0		32.8	562	377	21.1		2.6		30.0
24				0.29	69.5	5.1								21.6	10.1	2.6		
27	270–115 / 150–70	Marked	20–0.5	0.47	27.6	5.6	29.4	11.68	8.0		29.5	521	365	16.0	10.3	2.7	2.6	49.8
28	150–125 / 100–85	Slight	1–0	0.97	75.3	9.5	34.5	17.75	5.8		30.0	500	362	19.1	11.4	2.6	2.4	40.0
29	195–118 / 120–90	Slight	20–0.1	0.40	85.4	8.7	28.5	9.31	5.7		20.1	549	335	27.7	10.7	3.1		45.5
31	160–130 / 100–80	Slight	32–0	0.48	58.7	5.6	34.5	18.87	8.1		28.5	523	383	26.7	10.7	2.8	2.7	47.3
31				0.87	79.0	3.5	30.3	14.94	5.9		52.3	512	385	20.0	10.5	2.9	2.8	
33	180–128 / 110–90	None	4.5–0	0.42	66.0	2.1	40.8	28.00	7.3		24.5	541	345	29.2	13.2	2.8	4.2	45.7

36	185–135 110–100	None	32.5–0.1	0.23	72.8	3.6	28.5		4.4	150	52.9		377	14.0	10.8	3.0		49.9
36				0.18	78.7	8.4		11.09					369	17.6	11.8	2.6	3.1	40.0
37	160–100 90–70	Slight	1–trace	0.50	64.1	13.7	40.0		7.3		15.5	577	333	24.7	12.0	3.0	2.8	47.8
38	150–120 110–90	Slight	1.25–0	0.30	81.1	8.5	60.0	10.27	5.3	300	16.8	594	353	16.5	11.4	3.4	4.5	50.0
40	195–115 120–70	Moderate	5–trace	0.59	66.0	5.4	35.3	7.01	5.3	125	49.8		283	21.0	10.9	2.5	3.9	45.5
40							37.5		4.0	133	33.5		289	28.6	10.1	2.8	3.0	45.0
41	190–108 110–70	None	2–0.2	0.23	74.3	11.9	28.5	7.95	3.5	165	25.2		316	22.0	10.3	2.9	4.3	50.0
41									4.7	133	33.8		305	21.0	11.1	3.3	4.9	44.5
50	200–115 110–75	Moderate	6–trace	0.67	62.4	7.6	35.3	11.21	3.1	122	46.2	561	317	18.1	9.8	3.0	3.5	50.0
50	Foetus 230–135												270	26.7	10.3	3.1	3.7	71.5
51	220–110 110–85	Moderate	8–trace	0.81	70.5	7.8	31.6	7.61	3.6	130	38.7	549	317	23.0	9.7	3.0	3.4	50.0
54	220–110 120–70	Marked	28–trace	0.57	81.4	11.3	31.6	14.02	4.0	150	43.1	501	305	35.6	10.9	2.3	3.3	55.5
55	170–115 110–65	Slight	15–0	0.46	76.5	10.9	35.5	14.61	4.7	190	29.8	504	268	32.0	11.2	2.3	4.0	45.4
55	Foetus 218–110						35.5	18.48	4.7	133	42.0	504	257	35.6	11.4	2.7	4.8	45.4
56	140–68	Marked	10–5	0.56	51.0	7.0	23.0	9.34	3.2	172	43.3		302	21.0	10.9	2.4	3.5	47.6

toxemias of pregnancy. The blood pressure and albumin at the end of three weeks, together with the patient's previous history, are used for this differentiation. At the return examinations, six weeks after delivery, we often find it necessary to revise the diagnosis. This clearly indicates that our method of diagnosis is not without error. The group designated as pre-eclamptic toxemia may not be a definite entity, but rather comprised of cases showing different degrees of nephritis or eclampsia. In many instances we are not even able to differentiate between nephritic toxemia and eclampsia. If by nephritic toxemia we designate a type of case where the kidneys are not able to carry the extra load of pregnancy, and yet function normally between pregnancies, then we must form a separate group for the cases with permanently damaged kidneys,—chronic nephritis which might or might not have resulted from a pregnancy. It is even conceivable that typical eclampsia may in some instances be superimposed on a chronic nephritis, or may follow what we call nephritic toxemia. In the group of nephritic toxemias with convulsions one is impressed with the absence of symptoms and signs usually associated with nephritic uremia.

The inorganic elements, as well as the various cation ratios, show very slight differences between the normal non-pregnant and normal pregnant woman. Generally speaking, there is perhaps a tendency toward a slight lowering of the calcium in pregnancy, but this is so slight as to be negligible.

Nephritic toxemia: The outstanding and most surprising feature in this group of cases is the lack of evidence of any nitrogenous retention. The ammonia nitrogen in the urine and the uric acid of the blood are slightly elevated, whereas the non-protein nitrogen of the blood remains within normal limits, except in very severe cases when a slight increase is noted. The ratio $\frac{\text{B. U. N.}}{\text{N. P. N.}}$ in this group shows the greatest increase over normal; as does also the ratio $\frac{\text{B. U. N.}}{\text{U. N. \%}}$

Pre-eclamptic toxemia: The ammonia nitrogen of the urine and the uric acid in the blood are also slightly elevated in this type of toxemia. The ratio $\frac{\text{B. U. N.}}{\text{N. P. N.}}$ is lower than in normal pregnancy, as has been previously reported from this clinic. This ratio is, therefore, of some value in differentiating between the nephritic and the pre-eclamptic type. The ratio $\frac{\text{B. U. N.}}{\text{U. N. \%}}$ is also definitely lower in pre-eclampsia.

Eclampsia: In this group we notice the most marked variations from the normal. The ammonia nitrogen in the urine and the uric acid in the blood show definite elevations. The CO_2-combining power may be within normal limits for a woman at term, but will decrease to exceedingly low levels in most cases where coma supervenes. Such low CO_2 values are usually encountered late in the disease and indicate a developing acidosis which calls for immediate symptomatic treatment. The most interesting and perhaps most significant changes noted are those occurring in the sugar and inorganic phosphorus values. Unfortunately, we failed to make routine sugar determinations in our first series of cases

TABLE VI

Ratios—normal non-pregnant

CASE NUMBER	$\frac{\text{B.U.N.}}{\text{N.P.N.}}$ %	$\frac{\text{B.U.N.}}{\text{U.N.}}$ %	$\frac{(Na)+(K)+(P)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)+(P)}{(Mg)}$	$\frac{(Na)+(K)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)}{(Ca)}$	$\frac{(Na)}{(Ca)}$	$\frac{(K)}{(Ca)}$	$\frac{(P)}{(Ca)}$
16	53.8	20.5	28.1	139.4	27.9	35.0	32.8	2.11	25.3
18	57.7	22.2	26.3	125.3	26.0	33.0	31.0	2.06	28.6
19	30.9	11.1	27.0	125.4	26.8	34.2	32.4	1.86	25.3
43	28.2	11.5	27.6	119.7	27.4	35.6	33.7	1.92	24.0
44	45.3	17.1	27.5	124.	27.5	35.4	33.1	2.3	25.6
47	48.9	19.1	24.9	113.	24.6	31.7	29.6	2.02	29.2

TABLE VII

Ratios—normal pregnancy

CASE NUMBER	$\frac{\text{B.U.N.}}{\text{N.P.N.}}$ %	$\frac{\text{B.U.N.}}{\text{U.N.}}$ %	$\frac{(Na)+(K)+(P)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)+(P)}{(Mg)}$	$\frac{(Na)+(K)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)}{(Ca)}$	$\frac{(Na)}{(Ca)}$	$\frac{(K)}{(Ca)}$	$\frac{(P)}{(Ca)}$
1	41.5	16.28	27.4	139.4	27.2	34.2	31.9	2.18	23.5
5	47.0	19.17	30.5	159.5	30.2	37.5	35.5	1.99	24.75
13	41.5	14.97	32.0	150.8	31.8	40.3	37.6	2.69	33.75
42	45.8	15.8	27.3	143.8	27.05	33.1	31 2	2.2	26.75
58	44.3	15.75	29.6	173.	29.3	35.4	33.4	2.01	30.4

TABLE VIII

Ratios—nephritic toxemia

CASE NUMBER	$\frac{\text{B.U.N.}}{\text{N.P.N.}}$ %	$\frac{\text{B.U.N.}}{\text{U.N.}}$ %	$\frac{(Na)+(K)+(P)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)+(P)}{(Mg)}$	$\frac{(Na)+(K)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)}{(Ca)}$	$\frac{(Na)}{(Ca)}$	$\frac{(K)}{(Ca)}$	$\frac{(P)}{(Ca)}$
2	53.4	20.7							
2					36.23	48.8	44.2	4.0	
2					34.0	44.6	40.6	3.95	
21	41.2	21.95			26.58	34.0	31.7	2.31	
21	46.8	46.9		143.7					
32	43.8	43.9		103.					
32	63.1	63.2	25.4	122.8	25.3	31.9	29.6	2.3	21.5
6	50.2	13.85	30.9	139.	30.7	39.4	37.4	2.42	26.4
7	43.47	16.8				38.2	35.7	2.45	
25	30.8	12.72	32.5	174.1	32.3	39.6	37.4	2.19	23.8
25	37.0	19.13			31.8	40.6	37.5	3.79	
34	74.5	29.9	19.58	102.	19.3	23.4	21.43	2.58	20.1
46	41.3	12.22	23.8	127.	23.5			1.71	26.5
52		7.1	24.4	117.	24.2			2.08	30.0
53		16.5	21.8	117.2	21.6			1.47	26.7
57		10.68	27.25	132.	27.0			2.62	

TABLE IX

Ratios—pre-eclamptic toxemia

CASE NUMBER	$\frac{\text{B.U.N.}}{\text{N.P.N.}}$ %	$\frac{\text{B.U.N.}}{\text{U.N.}}$ %	$\frac{(Na)+(K)+(P)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)+(P)}{(Mg)}$	$\frac{(Na)+(K)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)}{(Ca)}$	$\frac{(Na)}{(Ca)}$	$\frac{(K)}{(Ca)}$	$\frac{(P)}{(Ca)}$
30	36.4	13.14	29.3	138.	29.1	42.7	40.4	2.16	23.2
3			30.9	132.	30.7	40.7	36.5	3.60	28.2
48	44.8	11.6	22.5	112.6	22.2	27.63	26.23	1.41	18.42

TABLE X

Ratios—eclampsia

CASE NUMBER	$\frac{\text{B.U.N.}}{\text{N.P.N.}}$ %	$\frac{\text{B.U.N.}}{\text{U.N.}}$ %	$\frac{(Na)+(K)+(P)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)+(P)}{(Mg)}$	$\frac{(Na)+(K)}{(Ca)+(Mg)}$	$\frac{(Na)+(K)}{(Ca)}$	$\frac{(Na)}{(Ca)}$	$\frac{(K)}{(Ca)}$	$\frac{(P)}{(Ca)}$
4						40.75	37.2	3.55	35.0
8	35.4	17.20	35.7	140.5	35.40	47.4	44.0	3.36	39.8
10								2.28	27.1
12						37.9	33.8	4.08	33.3
20	68.8	29.20	24.5	144.5	24.30		27.0	2.22	29.2
20			25.6	138.0	25.30		28.8	2.52	34.9
23	37.2	19.60	25.4	90.2	25.20	35.1	33.6	1.49	23.4
23	36.9	15.60				34.6	32.3	2.26	
24	55.0	23.10		285.0					
24								2.14	
27	39.7	42.40	29.5	142.0	29.30	37.0	35.4	1.56	25.2
28	51.4	23.60	27.4	147.5	27.20	33.4	31.8	1.68	21.0
29	32.6	10.82			26.20	33.9	31.3	2.59	
31	54.7	32.20	30.6	147.3	30.38	38.2	35.8	2.49	25.2
31	49.4	18.90	30.4	140.8	30.22	38.5	36.6	1.90	26.6
33	68.7	42.50	23.7	130.5	23.40	28.3	26.4	2.21	32.0
34					28.38	36.2	35.0	1.30	26.0
36			27.0	149.8	26.81	32.8	31.3	1.40	26.0
37	27.7	17.30	24.0	120.1	23.80	29.0	27.8	2.06	23.4
38	17.1	12.65	25.3	110.0	25.00	32.4	30.9	1.45	39.4
40	19.7	10.60	23.0	163.0	22 70	27.9	26.0	1.93	35.8
40		10.70	24.8	150.0	24.60	31.4	28.6	2.83	29.9
41	27.9		26.0	118.2	25.60	32.8	30.7	2.14	46.6
41			23.0	100.3	22.60	29.4	27.5	1.89	44.1
50	31.8	18.00	26.5	113.0	26.20			1.85	
50			22.4	97.2	22.20			2.59	
51	24.2	10.80	27.0	114.3	26.80	35.1	32.7	2.37	35.1
54	44.4	17.25	26.0	149.3	25.80	31.9	28.0	3.27	30 3
55	41.2	19.10	22.6	132.5	22.20	26.8	23.9	2.86	41.1
55 (foetus)	52.0		21.1	110.0	20 80	25 7	22 6	3.12	42.2
56	40.6	18.35	24.58	136.0	24 30	29.6	27.88	1.93	32.1

TABLE XI

Averages

	T.N.%	U.N.%	NH_3N%	N.P.N.	B.U.N.	$Ur.A_{cc}$	SUGAR	CO_2	NaCl	N_a	K	Ca	Mg	P	Fe	B.U.N./N.P.N.	B.U.N./U.N.%	$\frac{(N_a)+(K)+(P)}{(Ca)+(Mg)}$	$\frac{(N_a)+(K)+(P)}{(Mg)}$	$\frac{(N_a)+(K)}{(Ca)+(Mg)}$	$\frac{(N_a)+(K)}{(Ca)}$	$\frac{(K)}{(Ca)}$	$\frac{(P)}{(Ca)}$
Normal non-pregnant..	0.656	79.3	3.40	29.9	13.3	5.3	105.0	56.3	526	316	20.2	9.9	2.75	2.60	51.50	44.10	16.92	26.9	124.	26.7	34.2	32.1	2.1	26.3
Normal pregnancy.....	0.606	77.5	3.02	28.7	13.2	6	98.0	44.7	494	325	21.2	9.6	2.30	2.70	50.00	44.02	16.39	29.4	152.	29.1	36.1	33.9	2.7	27.8
Nephritic toxemia.....	0.390	75.1	5.57	29.6	12.7	5.4	100.0	46.8	505	331	24.4	10.3	2.77	2.87	49.40	47.78	23.97	25.7	128.	27.7	37.8	35.1	2.6	25.0
Pre-eclampsia.....	0.563	72.3	6.35	26.6	9.1	9	4.8	99.0	47.9	525	317	23.9	10.2	2.77	2.28	34.50	40.60	12.37	27.6	127.	27.3	37.0	34.4	2.4	23.1
Eclampsia.....	0.490	68.5	7.35	33.3	13.4	9.5	5.7	158.6	35.4	531	332	21.3	10.7	2.83	3.51	47.00	40.8	20.49	25.8	136.	25.8	33.5	31.0	2.3	32.4

Note: The ratio $\frac{[P]}{[Ca]}$ is expressed as a percentage.

and consequently blood sugar values are available for only the last nine cases. All of these show a definite, and in some cases quite a marked, hyperglycemia. On the average an increase of about 60 per cent over normal is observed.

Case 55 is of still further interest. In this instance the child was born after the mother had definitely improved, and its blood sugar was 133 mg. per 100 cc. of blood, whereas that of the mother was 190 mg. shortly after a convulsion. As we know that the blood sugar on the two sides of the placental membrane must be almost in equal concentrations, this observation indicates that in eclampsia the blood sugar increases with the severity of the case, and diminishes as improvement occurs. Our other figures also tend to lead to a similar conclusion.

Among the figures for the inorganic elements the most striking variations are found in phosphorus; the average value being 3.5 in eclampsia as against 2.7 in normal pregnancy. The ratio $\frac{[P]}{[Ca]}$ is also definitely increased, being 32.36, as compared with an average of 27.83 for normal pregnancy.

DISCUSSION

A study of the dispensary records, the hospital histories and the blood pressure columns of our tables, has impressed us with the importance of the diastolic pressure. In many cases the diastolic pressure is an earlier and surer indication than the systolic of an impending or developing toxemia; and even in the later stages of the disease this pressure is often the more reliable as an index of the severity of the case. That the diastolic pressure may give us an idea of the filtration pressure required for diffusion through the cell membrane has already been pointed out by one of us, and this is of special interest in connection with changes in cell permeability.

About fifteen years ago Jacques Loeb began his work on the "general salt effect" on a membrane. He showed that the diffusion of KCl through the egg membrane of Fundulus depends not only on the osmotic pressure of the KCl solution but that in addition a certain modification of the membrane must be brought about, which is perhaps produced by the salts upon the protein of the membrane. Hamburger and his co-workers have also attacked this problem of permeability and their general conclusions are that the Na-ion and K-ion have a softening effect, and the Ca-ion a condensing effect on the cell membrane. That irritability can be increased or decreased by changes in concentration of certain of the cations is now well known.

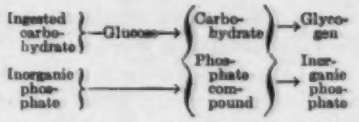
One cannot discuss changes in permeability in the living organism without thinking of changes in irritability. Indeed casual clinical observations would lead one to assume that the nervous irritability in eclampsia is quite different from that in the normal, and very probably increased. If this be the case, it may be permissible to assume that such a change in irritability may be the outcome of a shifting in the cation concentrations, and it is for this reason that we have been anxious to examine the inorganic ratios. The only significant change we have thus far noted is in the $\frac{[P]}{[Ca]}$ ratio.

Gross and Underhill found an increase in this ratio in parathyroid tetany. If analyses on a further series of cases corroborate our findings for this ratio, it would appear rational to assume that the use of $CaCl_2$ might be beneficial in eclampsia.

Benthin, Walthard, Obata and Hayashi, and others have shown a rise in the blood sugar in certain cases of eclampsia, and all our sugar values in that condition are definitely above the upper limit for normal. It is, of course, important that one differentiate between antepartum and parturient values, because labor itself induces a rise in blood sugar.

Langfeldt supposed that hyperglycemia may arise in any one of three ways. First, by a change of the hydrogen-ion concentration of the liver tissue; secondly, by a displacement of the curve of action of the liver diastase in such a way that the optimum would correspond or be close to the pH of the tissue; and lastly, by a lack in the formation of glycogen as the result of insufficient functioning of the pancreas. It is not probable that the hyperglycemia of eclampsia is due to the convulsion (muscular work) itself, because the elevated blood sugar persists for days after the last convulsion. Glycogenolysis is undoubtedly governed by the hydrogen-ion concentration in the liver cells; consequently, it would seem probable that the change producing hyperglycemia in eclampsia has taken place in the liver.

Recently Harrop and Benedict published the results of their work on the participation of inorganic substances in carbohydrate metabolism and have presented the following scheme:



The increase in both sugar and inorganic phosphorus in eclampsia may be quite significant from this viewpoint. It may also be well to bear in mind that adrenalin can cause hyperglycemia. It has been shown by Briggs, Koechig, Dorsy and Wener (1925) that insulin, in addition to causing a decrease in the sugar concentration, also brings about a lowering of the inorganic phosphate and potassium content of the blood of normal animals. In eclampsia we find a tendency towards an increase in these substances so that the therapeutic use of insulin in eclampsia may not be irrational, although we have used it mainly to combat the acidosis developing in seriously ill patients.

SUMMARY

We have discussed the possibility of a change in permeability resulting in an alteration in nervous irritability in eclampsia. It seems probable that such a change in cell permeability may be brought about by a variation in some of the cation ratios, such as $\frac{[Na]}{[Ca]}$, $\frac{[K]}{[Ca]}$, $\frac{[P]}{[Ca]}$ and others and we are of the opinion that alterations in these various ratios are associated with the problem of fetal nutrition.

The hyperglycemia consistently noted in eclampsia is probably due not to muscular activity, but rather to a change in the liver cells, such as a variation in their hydrogen-ion concentration. Moreover, it may be significant that both the sugar and the

inorganic phosphorus content of the blood are elevated in eclampsia.

We have discussed further changes noted in the urine and blood analyses of patients suffering from the late toxemias of pregnancy.

CONCLUSIONS

1. In *normal pregnancy* there is a slight decrease in the non-protein nitrogen of the blood and in the blood urea nitrogen. The CO_2-combining power is decidedly lower in pregnancy than in the normal non-gravid state. In normal pregnancy the inorganic elements are within the limits for the normal non-pregnant woman.

2. In *nephritic toxemia*, the most marked changes are noted in the ratios $\frac{\text{B. U. N.}}{\text{N. P. N.}}$ and $\frac{\text{B. U. N.}}{\text{U. N. \%}}$, both of which show an increase. The inorganic elements are within the limits for normal pregnancy.

3. *Pre-eclamptic toxemia* is accompanied by no outstanding variations except a slight decrease in the $\frac{\text{B. U. N.}}{\text{N. P. N.}}$ and $\frac{\text{B. U. N.}}{\text{U. N. \%}}$ ratios.

4. In *eclampsia* the following marked variations from the normal are noted:

a. An elevation in the uric acid of the blood.

b. A decrease in the CO_2-combining power, which is in proportion to the intensity of coma.

c. An elevated blood sugar, which remains above normal for an appreciable time after the cessation of the convulsion.

d. An increase in the ratio $\frac{[P]}{[Ca]}$, which is due to a high inorganic phosphorus value. The latter changes as well as those noted in the sugar suggest some association between carbohydrate and phosphorus metabolism, as well as a change in cell permeability.

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Multiple Osteomyelitis Due to Bacillus Paratyphosus B

Demonstration of the Bacillus in a Fresh Blood Preparation; Report of One Case[1]

By George L. Carrington, *Durham, N. C.*, and Wilburt C. Davison, *Baltimore, Md.*

A COLORED boy, aged 8 years, was admitted to the Harriet Lane Home on February 14, and discharged July 23, 1921.

The *family history* was without interest. The *past history* was unimportant except that the patient had had a large abdomen since birth and a large, hard and movable spleen had been felt in July, 1920, eight months before the present illness. For this no adequate explanation had been found.

Present illness. The date of onset was not definite. The patient had been feverish and short of breath for several months, had vomited frequently and had had pain in the abdomen, head and back. He had had frequent hemorrhages from the nose, and his legs and joints occasionally had become swollen and painful. The severity of his symptoms had apparently increased since July 5, 1921, six weeks before admission.

Physical examination (February 14, 1921). The temperature was 100°F. on admission but rose to 106°F. seven hours later. The pulse on admission was 124, the respirations 48. *Lungs.* The percussion note was impaired and the breath sounds diminished in the right axilla. *Abdomen.* The liver and spleen were definitely enlarged and both extended 5 cm. below the costal margin. The spleen was hard and smooth. *Extremities.* The right elbow and the lower end of the right humerus were slightly swollen and tender. The axillary and cervical glands were slightly enlarged, the breath was foul, the teeth were carious, the mucous membranes pale, the costochondral junctions and parietal bosses prominent. The remainder of the physical examination was essentially negative.

Laboratory examinations. The Wassermann reaction was negative. *Blood.* R.B.C. 3,064,000; some aniso- and poikilocytosis; many nucleated R.B.C.; platelets apparently increased; Hb. 36 per cent (Sahli); W.B.C. 19,800; differential count: P.M.N. 80 per cent, L.M. 12½ per cent, S.M. 6½ per cent. An intradermal tuberculin test with 0.1 mgm. O.T. was negative but with 1.0 mgm. was positive. *Roentgenological examination* revealed a shadow at the base of the right lung, believed to be due to pneumonic consolidation. The right humerus appeared normal.

Because of the enlarged spleen and the possibility of malaria, a *preparation of fresh blood was examined and two actively motile bacilli were seen.* A blood culture made immediately afterward contained B. paratyphosus B.

Course in hospital

2/17/21. The patient vomited 20 cc. of bright red blood, and complained of pain in the chest. The abdomen was slightly rigid and tender. W.B.C. 9800.

3/6/21. The temperature gradually fell from 106° to 101°F. between 2/17/21 and

[1] From the Department of Pediatrics, The Johns Hopkins University and the Harriet Lane Home, The Johns Hopkins Hospital, Baltimore, Md. Received for publication, Mar. 31, 1925.

3/3/22, then rose to 103° and continued between 100° and 103.6° F. The abdominal pain had gradually disappeared. The patient appeared to be more prostrated. The tenderness and swelling along the upper part of the right radius suggested osteomyelitis. The W.B.C. were 6800. Stool and urine cultures were negative, but on 3/2/21 the patient's serum had agglutinated B. paratyphosus B. in a dilution of 1 to 40.

3/12/21. The temperature varied between 100° and 103°F. The fusiform swelling over the lower end of the shaft of the right humerus had increased in size and was soft and somewhat fluctuating. It appeared to be definitely connected with the bone. About 2 inches below the right

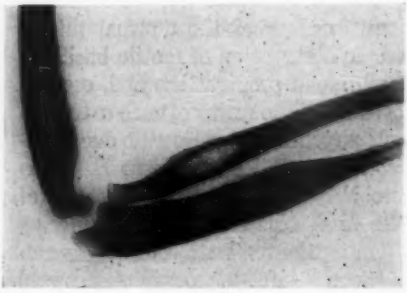

FIG. 1. RADIOGRAPHIC EVIDENCE OF OSTEOMYELITIS AND PERIOSTITIS OF THE RIGHT HUMERUS, RADIUS AND ULNA

elbow, on the flexor surface of the forearm there was another diffuse and somewhat soft swelling, which also appeared to be connected with the bones. Both swellings were tender. The upper one was aspirated and a small amount of fluid was obtained from which *B. paratyphosus B was isolated.* A blood culture three days later was sterile.

3/17/21. Roentgenological examination of the right arm revealed osteomyelitis and periostitis of the right humerus, radius and ulna (Fig. 1).

3/23/21. B. paratyphosus B was isolated from a stool culture. The swellings on the right arm had gradually increased in size and were definitely fluctuating. They were incised and pus was obtained. B. paratyphosus B was isolated from the pus. The

FIG. 2. RADIOGRAPHIC EVIDENCE OF OSTEOMYELITIS OF THE RIGHT FIBULA

lower end of the humerus appeared partially necrotic but the bone was not opened.

4/7/21. Fever (from 100° to 102°F.) continuing, the wound was widened and the lower end of the right humerus curetted. A sequestrum 2 cm. in length involving the entire thickness of the humerus was removed. The upper ends of the ulna and radius were incised and pus was obtained, but no sequestrum was found.

4/11/21. Swelling and tenderness appeared over the upper third of both fibulae and of the left ulna. Roentgenological examination revealed osteomyelitis in all

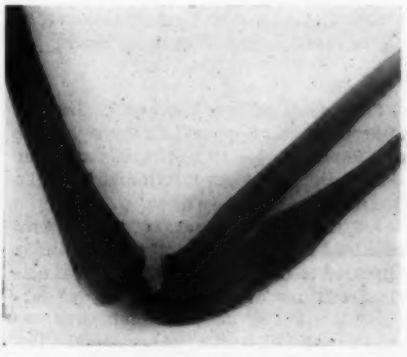

FIG. 3. RADIOGRAPHIC EVIDENCE OF OSTEOMYELITIS OF THE LEFT ULNA

three places (Figs. 2 and 3). These areas were incised and drained. B. paratyphosus B was isolated from the pus obtained.

4/28/21. Fever (from 100° to 103°F.) continuing, the right elbow was again incised and sequestra from the right radius and ulna were removed. Irrigation of this area with Dakin's solution was begun.

5/4/21. The temperature remained between 100° and 103°F. The patient complained of pain in the chest. Roentgenological examination revealed osteomyelitis of the left scapula and of all the ribs from the third to the ninth, inclusive, on both sides (Fig. 4). These had appeared to be normal on 2/15/21. The bones of the skull did not appear to be implicated.

FIG. 4. RADIOGRAPHIC EVIDENCE OF OSTEOMYELITIS OF THE THIRD TO NINTH RIBS ON BOTH SIDES

6/21/21. The fever continued. Lesions of herpes zoster appeared on the right side extending from the spine to the sternum at the level of the third, fourth and fifth ribs and disappeared 7 days later.

7/23/21. For two weeks the temperature remained under 100°F., and as the patient appeared to have completely recovered and stool cultures were negative, he was discharged with instructions to return at frequent intervals for observation. The spleen was still enlarged and hard.

2/21/22. The patient except for epistaxis two months previously had been in good health and had attended school. However, there was still a small sinus at the right elbow through which a little bloody pus was occasionally discharged. Roentgenological examination revealed that all the bones with the exception of the right elbow had become practically normal. The right elbow was much improved. The spleen was still enlarged.

2/16/23. The patient did not return for further observation. The Board of Health reported that he had died of "acute peritonitis" on this date, but no medical information was available and an autopsy was not performed.

This case is reported for two reasons, first, because of the unusual microscopic observation of motile bacilli in fresh blood preparations and, second, because of the rarity of such extensive osteomyelitis and periostitis due to infection by B. paratyphosus B. It is probably true that if preparations of the fresh blood of patients suffering from septicemia were routinely examined, the infecting organisms would occasionally be seen but, apparently, very few such observations have been recorded (1). Osteomyelitis and periostitis of one or two bones are not unusual complications of typhoid and paratyphoid fever, but as far as can be determined from the literature, no case has been reported in which the right humerus, radius and ulna, both fibulae, the left ulna and scapula and the third to ninth ribs on both sides were successively implicated at intervals during a period of two months.

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The Bile-Pigment Content of the Splenic Vein

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THE question of the relation of the spleen to haemoglobin metabolism is one which has had a place in the literature for years. That this organ is so very rich in blood, that erythrophagocytosis is so prominent within it under pathological conditions in man (and under normal conditions in many animals), that the spleen becomes enlarged during the course of certain diseases which appear to cause excessive red-cell destruction, and, finally, that at least two of these diseases may be arrested by splenectomy, are facts which speak for the importance of this organ in the metabolism of haemoglobin. The actual rôle of the spleen in haemoglobin metabolism is, however, quite obscure. Since haemoglobin can be readily broken down into simpler substances within the phagocytic reticulo-endothelial cells of the sinuses and pulp, it is clear that the organ is active in haemoglobin katabolism in those cases in which marked erythrophagocytosis can be demonstrated within it. But are these "simpler substances" identical with the *normal* products of haemoglobin katabolism? Certainly, in the case of the iron-containing pigment "haemosiderin," which is frequently found deposited in this organ, we have to do with an abnormal product of haemoglobin destruction, for the iron of the haemoglobin molecule is not normally precipitated in the tissues in the form of pigment. As for the soluble, and usually invisible, iron-containing product resulting from the breaking down of haemoglobin within the reticulo-endothelial phagocytes, it is impossible at present to be sure whether or not that is identical with the normal iron-containing product of haemoglobin katabolism, for we do not yet know enough of the nature of the latter substance. On the other hand, we are quite familiar with the properties of at least one of the normal *iron-free* products of haemoglobin katabolism—bilirubin; and, obviously, evidence that this pigment can be produced within the spleen has an important bearing on the question whether the spleen is able to effect the break-down of haemoglobin into its normal katabolic products.

In 1915, van den Bergh and Snapper (1) compared the bilirubin content of the splenic vein blood with that of peripheral venous blood in six cases of "haemolytic" anaemia. In three of these cases, the blood was drained

from the spleen after removal of the organ at operation; in the other three cases, the blood was obtained from the vessels at autopsy from one to several hours after death. In one of these autopsy cases the blood was taken from the portal vein instead of from the splenic vein. In five of the six cases, the splenic vein blood contained more bilirubin than the blood of the peripheral veins. Van den Bergh and Snapper presented these results as evidence that bilirubin can be formed within the spleen. Kaznelson (2), in 1920, confirmed their observation in a case of hemolytic icterus; but, on the other hand, Rosenthal (3) found no difference between the amount of bilirubin in the blood taken from the splenic artery and vein at operation in a case of haemolytic jaundice, nor did Lepehne (4) find any difference between the bilirubin content of the splenic vein and that of a peripheral vein in a case of so-called Banti's disease in which there was anaemia, splenomegaly, cirrhosis of the liver, and jaundice.

In view of the importance of the question, and of the few attempts which have been made to study the matter during the ten years which have elapsed since van den Bergh and Snapper's report appeared, we have carried out estimations of the bilirubin content of the splenic vein blood in various pathological conditions. In five of our cases the blood was obtained by aspiration of the vessels during operations for splenectomy. In the remaining five cases, the blood was removed from the vessels at autopsy shortly after death. In these latter cases, the splenic vein was clamped near its entrance to the portal vein before withdrawal of the blood, in order to prevent aspiration of the portal blood. In both the operative and the autopsy cases especial care was taken to handle the spleen very gently, or when possible, not at all, before blood was withdrawn from the vessels.

The bilirubin content of the various samples of blood was estimated by the van den Bergh quantitative "indirect" method, and in several of the cases Thanhauser and Anderson's modification of this test was also used.

CASES

A. White male; aged 59 years.

Typical case of pernicious anaemia, clinically and pathologically. R.B.C. 425,000; aniso- and poikilo-cytosis; Hb. 15 per cent; W.B.C. 5400; low platelet count; urobilinuria; lemon yellow color of skin.

Blood was aspirated from the splenic vein, from the axillary vein, and from the left ventricle at autopsy one hour after death. The bilirubin content of all three samples of blood was higher than normal, and *the serum from the splenic vein was found to have a bilirubin content nearly twice as great as that from the axillary vein or from the heart.*

B. White female; aged 53 years.

Typical case of pernicious anaemia, clinically and pathologically. R.B.C. 400,000; Hb. 17 per cent; W.B.C. 4200; low platelet count; urobilinuria; lemon yellow tint to skin.

Blood was aspirated from the splenic vein, the axillary vein, and the left ventricle one hour after death. The bilirubin content of all three samples of blood was higher than normal; *there was no difference in the amount of bilirubin in the different samples.*

C. White female; aged 14 years.

Clinically, a case of congenital haemolytic jaundice. R.B.C. 3,680,000; anisocy-

tosis, poikilocytosis; Hb. 54 per cent; W.B.C. 11,000; increased fragility of red cells; jaundice; urobilinuria; splenomegaly.

Blood was aspirated from the splenic artery and from the splenic vein at operation before the spleen was removed. A sample of blood was taken from an arm vein some minutes later. All three samples of blood contained more bilirubin than normally; but *the blood from the splenic vein contained just twice as much bilirubin as that from the splenic artery or from the arm vein.*

D. White female; aged 18 years.

Clinically and pathologically, a case of extreme chronic nephritis with severe secondary anaemia. R.B.C. 1,000,000; Hb. 20 per cent; W.B.C. 8000.

Blood was aspirated from the splenic vein, from the axillary vein and from the left ventricle at autopsy one hour and a half after death. The bilirubin content of all three samples of blood was quite low, and *there was no difference in the amount of bilirubin in the different samples.*

E. White male; aged 51 years.

Pathologically, a case of sarcoma of the mediastinum. There was a secondary anaemia with 3,225,000 R.B.C., Hb. 55 per cent and W.B.C. 6000.

At autopsy one hour and a half after death, blood was withdrawn from the splenic vein, from the axillary vein and from the left ventricle. The blood from the heart and from the axillary vein had a normal bilirubin content. *The blood from the splenic vein contained nearly twice as much bilirubin as did that from the heart and the arm vein.*

F. Colored male; aged 60 years.

A case of carcinoma of the stomach with widespread metastases and a mild secondary anaemia.

Blood was obtained for examination during an exploratory laparotomy. *The blood from the splenic vein was found to contain about three times as much bilirubin as arterial blood taken at the same time.*

G. White male; aged 34 years.

A case of severe secondary anaemia of undetermined nature. R.B.C. 2,400,000; anisocytosis and poikilocytosis; Hb. 30 per cent; W. B.C. 3100; no urobilinuria; R.B.C. resistance normal; free HCl in stomach contents; moderately enlarged spleen.

Blood was obtained from the splenic artery and vein at operation for removal of the spleen. *The bilirubin content of both samples of blood was identical.*

H. White female; aged 26 years.

A case of mental disease in which the blood was quite normal except for a slight leucocytosis before death.

Blood was withdrawn from the splenic vein and from the axillary vein at autopsy less than two hours after death. *The bilirubin content of both samples of blood was normal and identical.*

I. White male; aged 4 years, 7 months.

Clinically and pathologically, a case of nodular cirrhosis of the liver, with splenomegaly, ascites, and secondary anaemia (Hb. 52 per cent; W.B.C. 9400). Urine negative for urobilin.

Blood was withdrawn from the splenic vein and from an arm vein during the operation for splenectomy. *The bilirubin content of both samples of blood was normal and identical.*

J. White male; aged 38 years.

A case of jaundice with enlargement of the liver, splenomegaly, and secondary anaemia (R.B.C. 3,000,000; Hb. 50 per cent; W. B.C. 16,700). The resistance of the red cells was normal. There was free HCl in the gastric contents. Bilirubin was present in the urine.

The serum gave a direct van den Bergh reaction for bilirubin.

At operation the bile-ducts were found to be free from obstruction. The surface of the enlarged liver was smooth. A small piece of liver tissue removed for microscopical examination showed distended bile canaliculi, minute foci of necrosis of liver cells, periportal and also diffuse infiltration with polymorphonuclear leucocytes and mononuclear cells, and beginning proliferation of fine strands of connective tissue from the periportal areas.

Blood was removed from the splenic vein and from an arm vein during operation (Dr. Hughson).

The bilirubin content of both samples of blood was much higher than normal, but the amount of bilirubin was the same in each.

The following table shows the results of these examinations.

TYPE OF CASE	CASES IN WHICH SPLENIC VEIN BLOOD HAD HIGHER BILIRUBIN CONTENT	CASES IN WHICH SPLENIC VEIN BLOOD DID NOT HAVE HIGHER BILIRUBIN CONTENT
Pernicious anaemia	A.	B.
Haemolytic jaundice	C.	
Secondary anaemia	E., F.	D., G., I., J.
Cirrhosis of liver		I.
Subacute hepatitis with jaundice		J.
Normal blood picture		H.

DISCUSSION

These cases confirm the observation of van den Bergh and Snapper that the blood of the splenic vein *may* contain considerably more bilirubin than the blood taken at the same time from peripheral veins, or, indeed, from the splenic artery itself. It seems necessary to regard this fact as very strong evidence that bilirubin can be made within the spleen. The only other possible explanation would be that bilirubin might be stored within the organ and discharged from it at certain times. Were this true, one would have to assume that the periods of discharge happened to coincide with the moment of withdrawing the blood in those cases in which the bilirubin content of the splenic vein was found to be high; furthermore, one would expect the bilirubin content of the splenic vein blood to be lower than that of the arterial blood during the periods of storing, and this has never been observed.

It is clear from these studies, as well as from the conflicting reports in the literature, that although this evidence of bilirubin formation within the spleen has been observed most frequently in cases in which increased red cell destruction was prominent (so-called "haemolytic anaemias"), it cannot be foretold in just which pathological cases the splenic vein blood will show a high bilirubin content. The mere fact of increased red blood cell destruction is, apparently, not sufficient to bring about this condition. Thus, van den Bergh and Snapper (1) found no difference in the bilirubin content of the splenic artery and vein in the haemolytic anaemia and jaundice produced by toluylenediamine, whereas if a similar haemolytic poison (phenylhydrazine) were used, the splenic vein did often contain more bilirubin than the splenic artery; and Rich (5) found no appreciable difference between the bilirubin content of the splenic artery and vein during the haemolytic anaemia and jaundice produced by intravenous injections of distilled water.

In the present cases, no relation could be determined between the degree or the stage of the anaemia and the presence of a high bilirubin content of the splenic vein; and, furthermore, a careful microscopical study showed that there was no relation whatever between the amount of erythrophagocytosis or deposition of iron pigment within the spleen and the amount of bilirubin in the splenic vein blood.

As for the rôle of the spleen in bilirubin formation under *normal* conditions, we have examined repeatedly the blood of the splenic artery and vein in

normal animals and have never found any difference in the bilirubin content; nor was any difference found in the single human case reported here in which haemoglobin metabolism was, apparently, normal. The fact that, in the dog, splenectomy does not appreciably influence the output of bilirubin from a bile fistula (Whipple and Hooper (6), Inlow (7)) not even in the presence of extreme intravascular haemolysis (Rich (5)), speaks against any very essential participation of this organ in the normal formation of bile pigment.[1] However, it is entirely possible that the spleen may form bilirubin under normal conditions and discharge it into the blood stream in amounts too small to be appreciated by our present tests. One must remember that, in the dog, bilirubin can never be detected in the plasma under normal conditions, but if its excretion by the liver and kidneys is prevented, the pigment may accumulate up to detectable amounts.

As for the manner in which bilirubin may be formed within the spleen, no more can be said than that which is applicable to the problem of the manner of formation of bile pigment in general, i.e., that the answer lies, in all probability, either in the reticulo-endothelial cells in which this organ is so rich, or else in some as yet undetermined extracellular mechanism.

[1] It is of interest that, following the removal of the spleen in the case of haemolytic jaundice (Case C) reported here, there was a most remarkable decrease in the bilirubin content of the plasma, so that it had fallen to the normal level within forty-eight hours; and it remained at the low limit of normal during the patient's convalescence in the hospital. Repeated examinations of the serum failed to confirm Rosenthal's observation (3) that, following splenectomy in a case of haemolytic jaundice, the serum bilirubin gave a 'direct' van den Bergh reaction instead of the usual 'indirect' reaction which is characteristic of this disease, nor did the urobilinuria give place to bilirubinuria, as he reported. It seems probable that Rosenthal's case may have been complicated by some circumstance besides splenectomy.

SUMMARY

The bilirubin content of the blood of the splenic vein has been compared with that of the blood of the splenic artery and peripheral veins. In four out of ten cases the blood from the splenic vein contained much more bilirubin than the control blood. This is evidence that bilirubin can be formed within the spleen.

In pernicious anaemia and in secondary anemia, the splenic vein blood may or may not contain more bilirubin than the blood of the splenic artery.

In a case of haemolytic jaundice the blood from the splenic vein contained much more bilirubin than that from the splenic artery or from a peripheral vein. Following splenectomy the bilirubin content of the plasma fell to normal within forty-eight hours.

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On the Question of the Formation of Bile Pigment from Haemoglobin by the Action of Enzymes

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BRIEFLY to define the present status of the problem of the manner in which bile pigment is made in the body, it can be said that, although we are still quite uninformed as to the exact site of origin of this pigment, we are, nevertheless, in possession of at least three very important facts relating to the problem: (*a*) bile pigment may be formed from the haemoglobin of extravasated blood in any tissue or serous cavity of the body; (*b*) the pigment can be formed within the spleen; and (*c*) the pigment can be formed in an animal from which the liver and all the other abdominal organs have been removed.

The manner in which bile pigment is formed in haemorrhages has been the subject of numerous investigations, the details of which will not be entered into here. It will suffice to state that practically all students of this matter, as well as textbooks dealing with it, are agreed that the conversion of haemoglobin into bilirubin in haemorrhages occurs entirely extracellularly. It is believed that when haemoglobin is ingested by the wandering phagocytes which accumulate in such large numbers about a haemorrhage, the iron-containing pigment haemosiderin is formed, but not bilirubin. The latter pigment is believed to be formed only when haemoglobin disintegrates in the tissues outside of living cells (1) (2).

On the other hand, Löwit (3), Minkowski and Naunyn (4), McNee (5) and others have drawn attention to the frequent occurrence of red cells, bile pigment and an iron-containing haemosiderin-like substance side by side within the phagocytic cells of the liver, spleen and bone-marrow of certain animals, and they believe that the haemoglobin of the ingested red cells may be broken down into iron-free bile pigment and an iron-containing residue within these cells. These cells are part of the so-called "reticulo-endothelial system" of phagocytes which are distributed so widely throughout the body (16) and which are well known to be specifically active in erythrophagocytosis.

Since the publication of McNee's work (5), the question of the possibility that the reticulo-endothelial cells are responsible for the formation of bile pigment has been the subject of much discussion and many experiments, but, in spite of suggestive evidence, it has not as yet been proved that they have this power. However, their presence in great numbers about blood extravasations in every part of the body

and their activity in ingesting and breaking down the red blood cells have long been recognized, and if the activity of cells of any sort be responsible for the production of bile pigment in haemorrhages, it seems most probable that it is these ubiquitous cells that are concerned.[1] If, on the other hand, bilirubin is formed extracellularly in haemorrhages, as is generally believed, the enzyme responsible for the conversion of haemoglobin into bile pigment should be present in all tissues fluids, since this reaction can occur in any tissue or serous cavity.

The knowledge that bilirubin can be made within the spleen is based on the fact that under certain conditions the blood of the splenic vein has a much higher bilirubin content than that of the splenic artery (9) (10). The supporters of the idea that the reticulo-endothelial system is concerned with the formation of bile pigment, point to the exceptional richness of the spleen in cells of this type; while others believe that the transformation of haemoglobin into bile pigment within this organ occurs extracellularly in the tissue fluids.

As for the appearance of bilirubin in the circulation of an animal from which the liver and all other abdominal organs have been removed (7) (8), here one has to reckon with the reticulo-endothelial cells especially of the bone-marrow, and also with the idea which has continually found expression in the literature since it was first suggested by Virchow (11) in 1847—that the pigment can be formed extracellularly in the plasma of the circulating blood.

In spite of the almost universal acceptance of the belief that bile pigment is formed from haemoglobin extracellularly in haemorrhages, and of repeated statements concerning the existence of an enzyme in the plasma capable of effecting this transformation in the circulating blood (Aschoff (12) and others even speculate upon the site of origin of this hypothetical enzyme), when one turns to the literature to examine the evidence upon which this belief is based, it is surprising to find that there is not a single experiment on record in which a systematic attempt has been made to demonstrate the existence of an enzyme of this nature. In fact the only description of an attempt in this direction which we have been able to find is a single experiment carried out by Leschke (13), in which blood was introduced into the subarachnoid space by lumbar puncture. The bloody spinal fluid was later withdrawn and divided into two portions. Red blood cells were added to one portion and then both were incubated in a thermostat. After incubation, the portion to which red blood cells had been added was said to contain more bilirubin than the other. Leschke's belief was that the introduction of blood into the subarachnoid space had stimulated the discharge into the spinal fluid of an enzyme having the power to transform haemoglobin into bilirubin. The description of this single experiment is very meagre and there are numerous obvious sources of error in

[1] In spite of frequent assumptions that fixed tissue cells of various types possess the power to form bile pigment from haemoglobin, there is no microscopic evidence of any sort pointing to the activity of any type of cell other than those of the reticulo-endothelial system in this connection.

such a procedure. Aside from any consideration of the method, we have been unable to detect any increase in the bilirubin content of an haemorrhagic spinal fluid kept for days under aseptic conditions.

There are, however, on record, experiments in which haemoglobin is said to have been converted into bilirubin by the action of the enzyme trypsin. Crofton (14) in 1902 reported that if trypsin were allowed to act on haemoglobin in the presence of dextrose, bilirubin would be formed. Hollis (15) in 1908 reported confirmation of this observation but stated that dextrose was not necessary for the reaction, for bilirubin appeared when haemoglobin was incubated with trypsin alone.

As the following experiments will show, we have not been able to corroborate the statement that bile pigment is formed when trypsin acts upon haemoglobin.

EXPERIMENTS

Haemoglobin and trypsin. Five hundred cubic centimeters of a 0.25 per cent solution of Merck's haemoglobin were placed in each of three flasks. To the first flask were added 5 grams of trypsin ("Holadin" —Fairchild Bros. and Foster, N. Y.) which had been tested and found active; to the second flask were added 5 grams of trypsin and 5 grams of dextrose; the third flask contained only the haemoglobin solution. The contents of all three flasks were made slightly alkaline to litmus, incubated at 37°C. and tested at various intervals for the presence of bilirubin (Tables I, II, and III). The tests used were the van den Bergh, the Huppert-Salkowski, and the Gmelin, and the sediments were examined for bilirubin crystals. The van den Bergh test in all the experiments mentioned in this paper was checked by means of the control solutions described in another communication (6).

The haemoglobin solutions containing the trypsin gradually became decolorized, but at no time during a period of twelve days could any trace of bilirubin be detected in either of the three flasks. As has been pointed out elsewhere (6), when a haemoglobin solution, after standing for some time under various conditions, is layered with nitrous acid (Gmelin test), a green ring may appear at the surface of contact of the two liquids. This color does not indicate the presence of bilirubin, for other more delicate tests are entirely negative and the full play of colors of the Gmelin test does not appear. In the present experiments a pink ring often appeared when the haemoglobin solutions to which trypsin had been added were layered with nitrous acid. The sensitive van den Bergh test remained, however, entirely negative and it was afterwards found that when our trypsin was allowed to act upon protein solutions other than haemoglobin, the same pink color would appear on subsequent treatment with nitrous acid.

Is there a bile pigment forming enzyme in the circulating blood? In view of the assumption that haemoglobin can be changed into bilirubin by enzyme action within the circulation (12), we have carried out experiments in an attempt to demonstrate *in vitro* the presence of a bile pigment forming enzyme. When haemoglobin is injected intravenously into a normal animal it is well known that the transformation of this haemoglobin into bilirubin begins quite promptly. In the dog the injection of a sufficient amount of haemoglobin is regularly followed by an increased output of

bile pigment from the liver, and by the appearance of the pigment in the urine. In this animal, as a rule, the plasma is kept free from bilirubin for some hours following the injection, owing to the rapid excretion of bile pigment by the liver and kidneys. If the conversion of haemoglobin into bilirubin actually occurs in the circulating blood under these conditions, it should be possible to demonstrate *in vitro* the presence in the plasma of the active enzyme during the period in which haemoglobin is being transformed into bile pigment.

A dog, after fasting for 24 hours preceding the experiment, was anesthetized with ether and 25 cc. of blood were withdrawn from the jugular vein into 5 cc. of a 2.5 per cent sodium citrate solution under aseptic precautions. This blood was laked in 75 cc. of sterile distilled water and the solution then made isotonic by the addition of NaCl. The filtered laked blood was then injected slowly into the jugular vein. One hour later the urine, which before the injection contained a trace of bilirubin (Huppert test), showed the presence of much haemoglobin and gave a very strong test for bilirubin. A van den Bergh test on the blood serum at this time was clearly negative. Fifty cubic centimeters of blood

TABLE I

Haemoglobin and trypsin

NUMBER OF DAYS	GMELIN TEST	VAN DEN BERGH TEST	HUPPERT TEST
3	Greenish ring	Negative	Negative
5	Orange pink ring	Negative	Negative
7	Orange pink ring	Negative	Negative
10	Orange pink ring	Negative	Negative
12	Orange pink ring	Negative	Negative

TABLE II

Haemoglobin, trypsin and dextrose

NUMBER OF DAYS	GMELIN TEST	VAN DEN BERGH TEST	HUPPERT TEST
3	Orange pink ring	Negative	Negative
5	Orange pink ring	Negative	Negative
7	Orange pink ring	Negative	Negative
10	Orange pink ring	Negative	Negative
12	Orange pink ring	Negative	Negative

TABLE III

Haemoglobin solution alone

NUMBER OF DAYS	GMELIN TEST	VAN DEN BERGH TEST	HUPPERT TEST
3	Green ring	Negative	Negative
5	Green ring	Negative	Negative
7	Green ring	Negative	Negative
10	Green ring	Negative	Negative
12	Green ring	Negative	Negative

were now withdrawn from the carotid artery under aseptic precautions into sterile citrate, and then laked with an equal volume of sterile distilled water. This blood was placed in a series of four tubes which contained buffer solutions of various hydrogen-ion concentrations.[2] Toluene was used as a preservative. The tubes were incubated at 37° and tested for bilirubin after 24 and 48 hours. At the end of the experiment the solutions were dialyzed against a small amount of distilled water and the pH determined colorimetrically. The solutions and sediments were then extracted with a small amount of warm chloroform. Occasionally the chloroform was found to be slightly yellow after the extraction, and when this occurred, the chloroform was evaporated, the residue taken up with alcohol and this tested for bilirubin (van den Bergh test). Since bilirubin is slightly soluble in toluene, this fluid was always examined carefully for the slightest tinge of yellow. It remained colorless throughout all the experiments in which it was used (Table IV).

TABLE IV

Haemoglobin and plasma taken during excessive bile pigment production

pH AT END OF EXPERIMENT	VAN DEN BERGH AFTER 24 HOURS	VAN DEN BERGH AFTER 48 HOURS	CHLOROFORM EXTRACTION
5.8	Negative	Negative	Negative for bilirubin
6.4	Negative	Negative	Negative for bilirubin
7.5	Negative	Negative	Negative for bilirubin
8.2	Negative	Negative	Negative for bilirubin

Since it may be that the conversion of haemoglobin into bilirubin involves primarily oxidative processes, any enzyme concerned in the reaction might be of the nature of an oxidase. Experiments were therefore carried out in which oxygen was supplied to plasma containing haemoglobin.

The experimental method was, in principle, the same as that used in the preceding experiment. Laked blood was injected intravenously into a dog and allowed to circulate for one hour, at the end of which time the urine contained a very large amount of bilirubin, showing that the mechanism concerned in bile pigment formation was in full play. Blood was now withdrawn under aseptic precautions and citrated. The presence of an abundance of free haemoglobin in the plasma, because of the injection of laked blood, rendered further laking unnecessary. The plasma was entirely free from bilirubin. Portions of this citrated blood were buffered at different hydrogen-ion concentrations and incubated at 37°C. Oxygen was bubbled through the solutions in the incubator at frequent intervals. At the end of eighteen hours tests for bilirubin were carried out and the hydrogen-ion concentration of the different portions was determined colorimetrically after dialyzing against distilled water (Table V). Another experiment was carried out in exactly the same manner except that, in addition to oxygen, small amounts of hydrogen peroxide were added to the solutions at intervals during 24 hours (Table VI). In no instance could bilirubin be detected at the end of any of the experiments.

Examination of extracts of spleen for bilirubin-forming enzyme. Since we were unable, by the above methods, to demonstrate the presence in the

[2] The buffer solutions used throughout these experiments were prepared by combining molar solutions of dibasic sodium phosphate and citric acid in different proportions according to the tables given by McIlvaine (17).

circulating blood of an enzyme capable of transforming haemoglobin into bilirubin, we next turned to an examination of the tissue fluids of the spleen. Since we know that bile pigment can be formed in and delivered to the circulating blood by the spleen, it seemed most probable that, if a bilirubin-forming enzyme exists in any organ, it would be present in demonstrable concentration in this one. Furthermore, since the spleen is so rich in reticulo-endothelial cells, it was of interest to examine an extract of these cells for the presence of such an enzyme.

The spleen was removed from a dog by a clean operation under ether anaesthesia. The splenic veins were ligated before the arteries in order to induce engorgement of the organ with blood. The spleen was then cut into small pieces and ground thoroughly with sand under aseptic precautions. One hundred cubic centimeters of sterile distilled water were mixed well with the pulp and allowed to stand for half an hour. The haemoglobin-stained fluid was then poured from the sand and centrifugalized to free it from cellular debris. The supernatant fluid was pipetted off, put into sterile flasks and incubated at 37°C. Various preservatives were used in different experiments. Thymol, tricresol and toluene were tried, but the last was found to be the most satisfactory. Our tricresol alone yielded a colored solution on treatment with the van den Bergh reagent. The solutions were tested at various intervals for the presence of bilirubin but the tests were always negative. Table VII represents a typical experiment. No bilirubin crystals were ever seen on microscopical examination of the sediments.

TABLE V

Haemolyzed blood taken from circulation during period of excessive bilirubin formation. Oxygenated during incubation

pH AT END OF EXPERIMENT	VAN DEN BERGH AFTER 18 HOURS	CHLOROFORM EXTRACTION
Below 4.8	Negative	Negative for bilirubin
5.1	Negative	Negative for bilirubin
6.2	Negative	Negative for bilirubin
7.5	Negative	Negative for bilirubin
8.3	Negative	Negative for bilirubin

TABLE VI

Haemolyzed blood taken from circulation during period of excessive bilirubin formation Oxygen and H_2O_2 added during incubation

pH AT END OF EXPERIMENT	VAN DEN BERGH AFTER 24 HOURS	CHLOROFORM EXTRACTION
Below 4.8	Negative	Negative for bilirubin
5.0	Negative	Negative for bilirubin
6.2	Negative	Negative for bilirubin
7.4	Negative	Negative for bilirubin
8.4	Negative	Negative for bilirubin

Experiments were next carried out in which the splenic extracts were adjusted to different degrees of acidity and alkalinity before being incubated with laked blood. The general procedure followed in obtaining the extract was the same as in the preceding experiment except that the ground-up pulp was extracted in the ice-chest for

a longer period of time. In the first of these experiments the haemoglobin-containing extraction solution was divided into two parts. To one part, acetic acid was added sufficient to make a 0.2 per cent acid solution; to the other, sodium carbonate was added in amount calculated to form a 0.2 per cent solution. After incubation at 37°C. the solutions were tested for bilirubin (Table VIII).

In the next experiments exactly the same procedures were employed except that the extracts were adjusted to different hydrogen-ion concentrations by adding 2 cc. of the buffer solutions

TABLE VII

Extract of spleen incubated with laked blood

TIME OF INCUBATION	VAN DEN BERGH TEST	GMELIN TEST	HUPPERT TEST	CHLOROFORM EXTRACTION
24 hours	Negative	Green ring	Negative	Negative for bilirubin
2 days	Negative	Green ring	Negative	Negative for bilirubin
3 days	Negative	Green ring	Negative	Negative for bilirubin
4 days	Negative	Green ring	Negative	Negative for bilirubin
5 days	Negative	Green ring	Negative	Negative for bilirubin

TABLE VIII

TIME OF INCUBATION	VAN DEN BERGH TEST	GMELIN TEST	HUPPERT TEST	CHLOROFORM EXTRACTION
Extract of spleen (acid) incubated with laked blood				
24 hours	Negative	Greenish ring	Negative	Negative for bilirubin
2 days	Negative	Greenish ring	Negative	Negative for bilirubin
5 days	Negative	Greenish ring	Negative	Negative for bilirubin No crystals microscopically
Extract of spleen (alkaline) incubated with laked blood				
24 hours	Negative	Greenish ring	Negative	Negative
2 days	Negative	Greenish ring	Negative	Negative
5 days	Negative	Greenish ring	Negative	Negative No crystals microscopically

TABLE IX

Splenic extract incubated with laked blood at different pH

pH AT END OF EXPERIMENT	VAN DEN BERGH AFTER 24 HOURS	VAN DEN BERGH AFTER 2 DAYS	VAN DEN BERGH AFTER 3 DAYS	VAN DEN BERGH AFTER 5 DAYS	VAN DEN BERGH AFTER 5 DAYS	CHLOROFORM EXTRACTION
Below 4.8	Negative	Negative	Negative	Negative	Negative	Negative for bilirubin
4.9	Negative	Negative	Negative	Negative	Negative	Negative for bilirubin
5.1-5.3	Negative	Negative	Negative	Negative	Negative	Negative for bilirubin
6.1	Negative	Negative	Negative	Negative	Negative	Negative for bilirubin
6.7	Negative	Negative	Negative	Negative	Negative	Negative for bilirubin
7.4	Negative	Negative	Negative	Negative	Negative	Negative for bilirubin
7.9	Negative	Negative	Negative	Negative	Negative	Negative for bilirubin

to 12 cc. of the haemoglobin-containing extracts. Table IX represents the composite results of this series of experiments.

The preceding experiments were next repeated with the added factor of a liberal supply of oxygen to the solutions during incubation. Oxygen was bubbled through the splenic-extract-laked-blood mixture at frequent intervals and, in some instances, small amounts (0.5 cc.) of hydrogen peroxide were also added. The results of these experiments are shown in Tables X and XI.

Finally, since it has been stated that haemoglobin can be transformed into bilirubin by incubation with autolyzing tissue, we have allowed splenic tissue to autolyze in contact with laked blood under sterile conditions and in the presence of air bacteria. No bile pigment was formed in either case (Table XII). Indeed, Hauser, who mentions the occurrence of "needle-shaped crystals of bilirubin" in the sediment which formed when blood was incubated with autolyzing tissue, made no attempt whatever to determine whether the crystals which he saw were really bilirubin crystals (18).

TABLE X

Spleen extract incubated with laked blood at different pH. Addition of oxygen

pH AT END OF EXPERIMENT	VAN DEN BERGH TEST AFTER 24 HOURS	CHLOROFORM EXTRACTION
Below 4.8	Negative	Negative for bilirubin
4.9	Negative	Negative for bilirubin
6.1	Negative	Negative for bilirubin
7.6	Negative	Negative for bilirubin
8.2	Negative	Negative for bilirubin

TABLE XI

Spleen extract incubated with laked blood at different pH. Addition of oxygen and H_2O_2

pH AT END OF EXPERIMENT	VAN DEN BERGH TEST AFTER 24 HOURS	CHLOROFORM EXTRACTION
Below 4.8	Negative	Negative for bilirubin
5.0	Negative	Negative for bilirubin
6.1	Negative	Negative for bilirubin
7.5	Negative	Negative for bilirubin
8.4	Negative	Negative for bilirubin

TABLE XII

Laked blood incubated with autolyzing spleen tissue

TIME OF INCUBATION	VAN DEN BERGH TEST	CHLOROFORM EXTRACTION	MICROSCOPICAL EXAMINATION	CONTROL CULTURE
2 days	Negative	Negative for bilirubin	No crystals, many bacteria	Mixed flora
4 days	Negative	Negative for bilirubin	No crystals	No growth

DISCUSSION

Although all of the attempts which we have made up to the present, to demonstrate the existence of a bile pigment forming enzyme, have been unsuccessful, it is obvious that we cannot conclude that an enzyme of this nature does not exist in the body, for there remain further methods which should be applied to the solution of this problem. However, we have in the present experiments supplied many of the ordinary conditions favorable to enzyme action, and the failure to obtain bilirubin from haemoglobin under these conditions shows at least that, if such an enzyme exists in the plasma or spleen, conditions of a different sort are required for its activity. In the light of the present status of the problem of the manner in which bilirubin is formed within the body, it seems important that an exhaustive search be made for the presence of this frequently postulated enzyme. It is obvious that its existence or non-existence is a matter of considerable import to the question of haemoglobin metabolism as well.

SUMMARY

The assumption is frequently made that there is present in the body an extracellular enzyme having the power to convert haemoglobin into bile pigment. We have been unable to demonstrate an enzyme of this nature in the plasma or in the spleen.

Contrary to statements in the literature, bilirubin was never formed in our experiments when haemoglobin was subjected to the action of trypsin.

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Book Reviews

International Clinics. Ed. by Henry W. Cattell, A.M., M.D., and Charles H. Mayo, M.D. [et al.] $2.00. (*J. B. Lippincott Co., Philadelphia, 1924.*)

Vols. III and IV of the 34th series contain the usual excellent articles of general medical interest. Public Health, Medical, Pediatric and Surgical Problems are discussed quite fully. Of especial interest are the articles by Zingher on "The Dick Test," Abbot and Dawson on "The Clinical Classification of Congenital Cardiac Disease" and Turner on "Calcification of the Pericardium." A very good general article on Insulin appears in Volume IV by Banting. On the whole the two volumes are quite worth while.

C. S. K.

Rheumatic Heart Disease. By CAREY F. COOMBS. Pp. 376. $4.50. *Wm. Wood & Co., New York, 1924.*)

In this volume we have an excellent presentation of the subject of Rheumatic Heart Disease, of the large personal experience of the author and of the course of rheumatic infection. The arrangement of the text is quite good and covers the subject from etiology to treatment. One is rather surprised that the author feels that rheumatic fever is due to a streptococcus infection. While some of the evidence which he brings forth in support of this belief is interesting, it seems best for the present, at least, to consider the cause of acute rheumatic fever as unknown. The illustrations in the text are excellent.

C. S. K.

The Serum Diagnosis of Syphilis by Precipitation. R. L. KAHN, M.S., D.Sc. 237 pp. $3.00. (*The Williams & Wilkins Company, Baltimore,* 1925.)

In view of the popularity of the author's test, his little book comes at a very opportune time. The steps whereby Dr. Kahn has arrived at his present technique have been carefully considered and controlled, and are clearly presented. His antigen—an alcoholic extract of beef heart reinforced with 0.6 per cent cholesterine—has the advantage over the Wassermann antigens in the manner in which it can be controlled during its preparation and before it is used. This is of great help in determining the amount of ether extractive substances that have been removed. The necessity of pipetting such small quantities as 0.0125 cc. of antigen dilution may be, however, a possible source of error. The author has also thought it necessary to include a quantitative procedure as of probable value in cases undergoing treatment. The necessity for such a test is still debatable.

The method of testing spinal fluid, which necessitates the precipitation of the protein of the fluid by the addition of ammonium sulphate, is rather complicated and might cause confusing results in the hands of inexperienced workers.

As an index of its clinical value, the author reports the results of 100,000 Kahn tests compared with those of the Wassermann reaction after one hour's incubation in the ice-box. This group includes 3600 tests in which the results have been tabulated according to the stage of syphilis in the different patients, and 1000 tests of the sera of patients undergoing antisyphilitic treatment. Over 2600 tests on non-syphilitic individuals have also been performed.

Although the proportion of agreement between the Kahn test and the Wassermann reaction is greater than with any precipitation test reported to the present time, the years of clinical experience which have served as a check on the Wassermann reaction have given it a prestige difficult for a test as recent as the Kahn test to overcome. At present it can only be used in conjunction with the Wassermann reaction as a possible aid in the diagnosis of syphilis.

J. E. K.

The Life of Sir William Osler. By HARVEY CUSHING. 2 vols. $12.50. (*Oxford University Press, New York.*)

Through the courtesy of the Oxford Press we have received a copy of Dr. Harvey Cushing's eagerly expected "Life of Sir William Osler." As there was not sufficient time to attempt any adequate and just review of these two volumes for the June number of the BULLETIN (such a review will appear later) we wish merely to call the attention of our readers to this work and to express the hope that those who read this extremely absorbing life story may find a complete fulfillment of the pleasure and inspiration afforded in even a hurried glance over the wealth of detail gathered into these two volumes.

A tremendous task, undertaken by Dr. Cushing at the request of Lady Osler, in a spirit of the finest loyalty and devotion to the memory of the much beloved Chief, he has accomplished his purpose in a most successful way. Weaving his story about the thread of Dr. Osler's letters, with a very human and sympathetic linking together of all the significant epochs and lighter incidents, he has succeeded admirably in creating the spirit and the atmosphere of that life which meant so much to so many and wielded, for all too short an earthly span, such an extraordinary influence upon the evolution of the study of medicine and upon mankind.

E. P. C.

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